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Journal of Chromatography A, 812 (1998) 343–367

JOURNAL OF CHROMATOGRAPHY A

Review

Analysis of fluoroquinolones in biological fluids by high-performance liquid chromatography

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Abstract

High-performance liquid chromatographic methods for the analysis of fluoroquinolones in biological fluids are reviewed. In particular, sample preparation and handling procedures, chromatographic conditions and detection methods are discussed. A summary of published high-performance liquid chromatographic assays for individual fluoroquinolones is included.
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Keywords: Reviews; Sample preparation; Fluoroquinolones

Contents

1. Introduction

The quinolones are synthetic antibiotics, chemically related to nalidixic acid, with which they share the same mode of action. These drugs form a group of antimicrobial agents with different chemical structures and spectra of activity. The general structure of quinolone antibacterial agents consists of a 1-substituted-1,4-dihydro-4-oxopyridine-3-carboxylic moiety combined with an aromatic or heteroaromatic ring (**1**) (Fig. 1).

Since the discovery of norfloxacin (**2**), extensive research in this area has been carried out. Almost all of the recent clinically useful quinolones bear a fluorine atom in the C-6 position of the quinolone, naphthyridine or benzoaxazine ring system.

Because of the presence of the fluorine atom in the molecules, these antibacterial agents are generally

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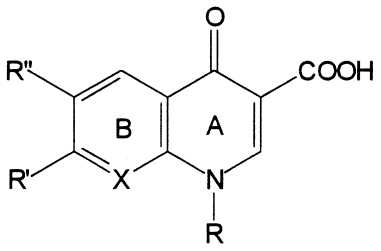
PII: S0021-9673(98)00274-X



(1)

Fig. 1. General structure of quinolones. X=C; R=cyclopropyl, ethyl, fluoroethyl, methylamino, fluorophenyl group and thiazine or oxazine ring. R′=piperazin-1-yl, 4-methylpiperazin-1-yl, 3-methylpiperazin-1-yl; R″=fluorine atom.

described as fluoroquinolones. Pharmaceutical research on the fluoroquinolones has made considerable progress in expanding their initial indication of treating urinary tract infection (UTI) to include systematic infections other than urinary tract infection.

Several recent derivatives have expanded their antibacterial spectrum to include activities against anaerobic bacteria. Moreover, the pharmacokinetic properties have been improved and adverse reactions, especially central nervous system (CNS) side-effects, reduced as well. The mode of action of these agents is better defined and they are now being considered by some as the potential drugs of choice for the treatment of a number of bacterial infections.

Attempts have been made to subdivide the quinolones into various generations based upon antibacterial spectrum, potency and pharmacology. To date, no classification has been accepted, although terms such as first-, second- and third generation have occasionally been used. First generation quinolones, e.g. nalidixic acid and oxolinic acid, may refer to agents which lack good anti-Gram positive activity and are predominantly used for the treatment of urinary tract infection.

The second generation quinolones, e.g. norfloxacin and ciprofloxacin, are those used for the treatment of systemic infections as well as for urinary tract infection and possess activities against both Gram-positive and Gram-negative bacteria.

The third generation quinolones, e.g. temafloxacin, include those having increased potency against Staphylococcus aureus as well as an expanded spectrum, including anaerobic bacteria, clamydie and mycoplasma. Other classifications based on pharmacokinetic or CNS profiles are also being made for commercial reasons. For example, difloxacin and fleroxacin are long-acting quinolones, while temafloxacin and tosufloxacin are quinolones with fewer CNS side-effects.

The structure–activity relationships, microbiology and synthetic chemistry associated with quinolones up to 1977 has been reviewed [1]. Due to explosive growth in this field in recent years, several reviews on the microbiological and clinical aspects of quinolones have been published [2–8]. Concise reviews of the structure–activity relationship of new fluroquinolones have also been published [9–12]. Several books on the chemistry, microbiology, pharmacology, modes of action and clinical uses of quinolones have been published [13], with the biological and clinical aspects being emphasized.

The target of quinolones in bacteria is DNA gyrase, a type II topoisomerase. DNA gyrase is an essential enzyme which is responsible for negatively supercoiling covalently closed circular DNA and also in catenation and decantation reactions [14]. The mechanism of action of the quinolone antibacterial agents involves the inhibition of DNA gyrase (a bacterial topoisomesare II), resulting in a rapid bactericidal effect. A discussion on this unique mode of action, will be presented by several authors [15–18].

Therapeutic drug monitoring of fluoroquinolones is most applicable when the drug in question has a narrow therapeutic range, is used chronically, has potentially toxic side effects if overdosed. Both clinical and molecular studies of the pharmacological profiles of a wide variety of fluoroquinolones have demonstrated that a much better correlation exists between the observed clinical effects of a fluoroquinolone and its plasma concentration than that observed between the clinical effect and total daily drug dosage.

Numerous techniques have been developed for the analysis of fluoroquinolones in biological fluids and pharmaceutical preparations. The analysis of fluoroquinolones has traditionally been performed using microbiological methods. However, these technique is slow and suffers from poor precision and specificity, since active metabolites and co-administered

antimicrobial may interfere. Although numerous chemical and physical techniques have been reported for the assay of fluoroquinolones, they suffer from a variety of disadvantages.

High-performance liquid chromatography (HPLC) in particular has become an important tool for routine determination of antimicrobial agents in body fluids [19–23]. The major benefits of HPLC are specificity, rapidity and sensitivity.

A further advantage from the pharmacokinetic point of view is its potential for the detection and quantitation of metabolic degradation products. Owing to the short time required for HPLC analysis and its great potential for the separation and detection of antibiotic drugs, many reports have focused on the techniques for the determination of various drugs in biological fluids [24–27].

This reviews extensively summarizes the most HPLC assay designed for the determination in biological fluids of some fluoroquinolones like norfloxacin, ofloxacin, ciprofloxacin, pefloxacin, amifloxacin, fleroxacin, difloxacin, lomefloxacin and rufloxacin.

2. Norfloxacin

Norfloxacin (**2**) (MK-366; AM-715) or 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid is an antimicrobial agents. Chemical formulae of norfloxacin (**2**) and of its three major metabolites: 7-(3-oxo-1-piperazinyl) (**3**); 7-[(2-aminoethyl)amino] (**4**); 7-[(2-acetylaminoethyl)amino] (**5**) are shown in Fig. 2.

Norfloxacin (**2**) was the first quinolone antibacterial with a fluorine atom substituted at the C-6 position and a piperazine at the C-7 [28]. It also represented the first significant increase in antibacterial activity over the previous quinolones with a spectrum that covered both Gram-positive and Gram-negative organisms including *Pseudomonas aeruginosa*.

The mode of action, in vitro activity, clinical efficacy and utility, and the pharmacokinetics and metabolism of this drug has been reviewed [29]. Several methods for quantitation of norfloxacin (**2**) have been published so far [30–38]. Studying these methods I concluded that quantification is possible

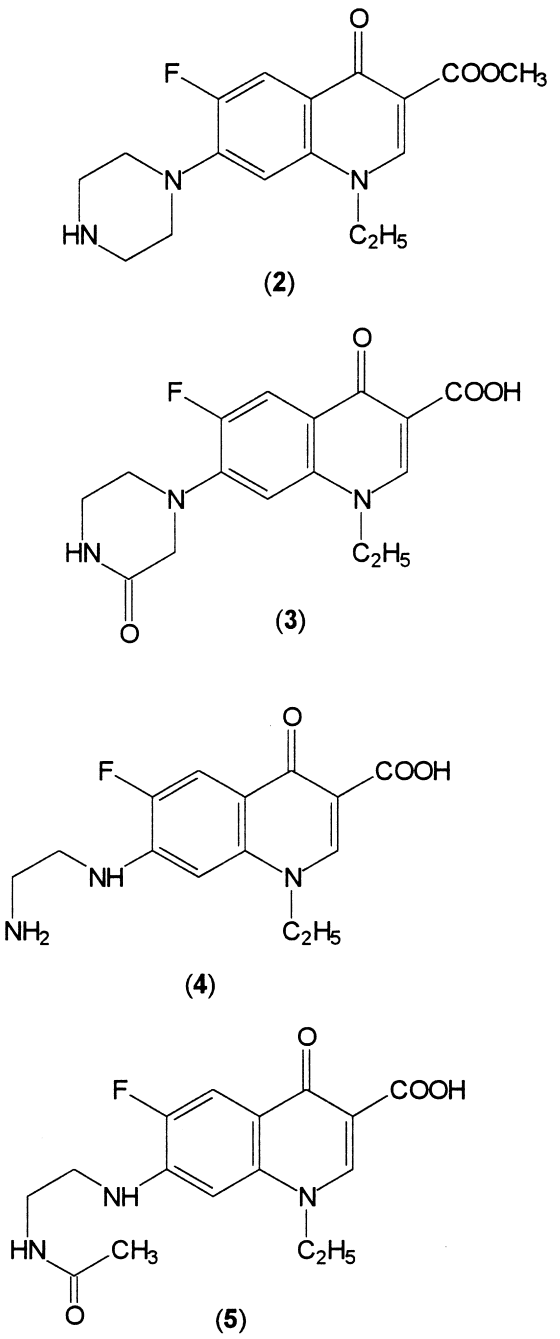


Fig. 2. Chemical structures of norfloxacin and its metabolites.

with a reversed-phase HPLC column, using an acidic eluent that consists of methanol or acetonitrile, water and an ion-pairing reagent of the tetrabutylam-

monium type, which reduces retention times of the protonated quinolones by competing for the available adsorption sites of the stationary phase, as described by Tilly Melin et al. [39].

Pauliukonis et al. [30] describes a reversed-phase ion-pair HPLC method for the quantitation of norfloxacin (**2**) in human plasma and urine, using pipemidic acid as the internal standard. The ability of this method to distinguish intact norfloxacin (**2**) from its metabolites was demonstrated. Norfloxacin (**2**) was extract from the sample matrix using dichloromethane under neutral conditions, followed by back extraction into dilute phosphoric acid for chromatographic analysis on a reversed-phase column (μBondapak C_{18}) with a mobile phase consisting of methanol, phosphate buffer, and ion-pairing reagent (pH=3.0). The assay for norfloxacin (**2**) in plasma utilized a fluorescence detector with excitation at 280 nm, and emission at 445 nm, while the urine assay used a UV absorbance detector set at 280 nm.

To assess the presence of norfloxacin conjugates in the urine of dosed individuals, the effects of urine hydrolysis on drug quantitation were examined. Gutzlel et al. [32] described a method for the determination of norfloxacin (**2**) in human plasma and urine using a solid-phase extraction (Sep–Pak cartridge C_{18}) for sample preparation, followed by HPLC with fluorescence detection. The limit of detection for norfloxacin (**2**) in plasma and urine was 0.02 $\mu g\,ml^{-1}$ and 0.5 $\mu g\,ml^{-1}$, respectively.

Laganà et al. [40] also developed an HPLC method with fluorescence detection for the analysis of norfloxacin (**2**) in human tissues and plasma. The analytical procedure in the tissue pretreatment consists of extraction using a Carbopack column. The samples were chromatographed on a C_8 reversed-phase column.

In this case a mobile phase was a mixture of acetonitrile–methanol–phosphate buffer (pH=2.5). The limit of detection for norfloxacin (**2**) was ca. 1 $ng\,ml^{-1}$. A HPLC method for the determination of norfloxacin (**2**) concentrations in body fluids was developed by Morton et al. [31] and compared with a standard bioassay. The assay utilizes a reversed-phase C_{18} column, an internal standard, and fluorescence detection, with reproducibility studies yielding R.S.D.s ranging from 0.6 to 3.7% for norfloxacin, while correlation coefficient with the bioassay was 0.966. Several other HPLC assays for norfloxacin (**2**) have been described [40–44], most have not incorporated an internal standard or have not been evaluated with specimens from patients on multiple drug regimes [30,41,42].

The HPLC method of Forchetti et al. [45] for norfloxacin (**2**) determination in serum, urine or tissue (either prostate or kidney) did not include an internal standard and required a 1 ml sample of serum or urine as well as multiple extractions with dichloromethane and back extraction with sodium hydroxide before analysis. The chromatographic separation was achieved in a Vydac 10 μm anion-exchange column, connected to an AXGU 10 μm anion-exchange precolumn. The mobile phase used was a mixture of acetonitrile–0.05 *M* phosphate buffer (pH=7.0). No interference was observed during the assay between norfloxacin (**2**), its major metabolite (**3**), and the endogenous substances in the clinical samples. Wallis et al. [46] developed a rapid and economical HPLC method for the determination of norfloxacin (**2**) in serum using a microparticulate C_{18} guard cartridge. Samples (100 μl) containing *N*-ethylnorfloxacin as the internal standard were extracted into 1 ml of chloroform. Chromatography was performed at 30°C on a 40×3.2 mm I.D. C_{18} guard cartridge (3 μm spherical particles) using a mobile phase of acetonitrile–0.01 *M* phosphate buffer (pH=2.5)–0.001 *M* trietylamine. Detection was at 279 nm.

A number of authors have shown that certain fluoroquinolones have a pharmacokinetic interaction with methylxantines, and non steroidal anti-inflammatory agent, due to metabolic inhibition [47,48]. Davis et al. [49] developed a HPLC method for the simultaneous assay of fluoroquinolone and theophylline in plasma. It involves extraction of plasma with chloroform–isopropanol prior to chromatography on a Spherisorb ODS2 column. Ultraviolet detection is carried out at 280 nm. Carlucci et al. [50] developed a simple HPLC method for the simultaneous determination of norfloxacin (**2**) and furprofen a non steroidal anti-inflammatory. Followed dichloromethane extraction, the solution was chromatographed in an anion-exchange column using a mobile phase of 0.01 *M* phosphate buffer (pH=7.0)–acetonitrile. The calibration curves for the two drugs in plasma were linear over the range 0.1–5.0 $\mu g\,ml^{-1}$ for

norfloxacin and 0.1–3.0 μg ml^{-1} for furprofen and the correlation coefficients of the calibration curves were 0.999 and 0.997, respectively. The lower limit of the method, defined as three times the level of the baseline noise, was 0.05 μg ml^{-1} for each drug.

Another HPLC method for the simultaneous determination of norfloxacin (**2**), fenbufen and felbinac, in plasma was developed by Katagiri et al. [51].

3. Ofloxacin

Ofloxacin (**6**) a new pyridone carboxylic acid derivative was synthesized by Hayakawa et al. [52]. Its chemical name is (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3,-de]-1,4-benzoxazine-6-carboxylic acid (HOE 280, DL-8280) (Fig. 3). Collections of papers describing the microbiological, pharmacological and clinical aspects of ofloxacin (**6**) have been published [53–55].

Sudo et al. [56] identified in various animal species two metabolites as follows: *N*-demethylated (**7**), and DL-8280 *N*-oxide (**8**).

Ofloxacin (**6**) is effective in treating a variety of acute and chronic infections, including simple and complicated urinary tract infections, respiratory tract infections and osteomyelitis [57,58]. Several high-performance liquid chromatographic methods have been reported for the analysis of ofloxacin (**6**) in biological fluids e.g. serum, plasma, urine, bile, bronchoalveolar lavage fluid, saliva, skin blisters, cerebrospinal fluid, scalp hair [59–88].

Some of these methods have utilized UV detection [60,61,64–66,88], while others have utilized fluorescence detection [62,67–71,75,78,79,84,87]. Some of these methods have not included internal standards [60,66,88]. Ofloxacin (**6**) concentrations in plasma and urine samples were determined by using a HPLC method by Le Coguic et al. [70]. Briefly, samples were analyzed by using a C_{18} Nova–Pak column (4 μm, 150 mm×3.9 mm I.D.) after extraction at pH 6.8 with dichloromethane.

The mobile phase consisted of a mixture of acetonitrile–0.01 *M* potassium dihydrogenphosphate–triethylamine. The pH was adjusted to 2.8 with formic acid. The 1-allyl-6-fluoro-7-(4-methyl-1-piperazinyl)-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid was used as an internal standard. The eluate was monitored fluorimetrically (307 nm excitation and 510 nm emission). The limit of detection for the urine and plasma were 9 and 0.5 μg l^{-1}. Some compounds often associated with ofloxacin (**6**) in therapeutic were tested for interference. The extraction procedure required a small volume of sample (100–500 μl) and was also simplified compared with the Notarianni et al. [69] method.

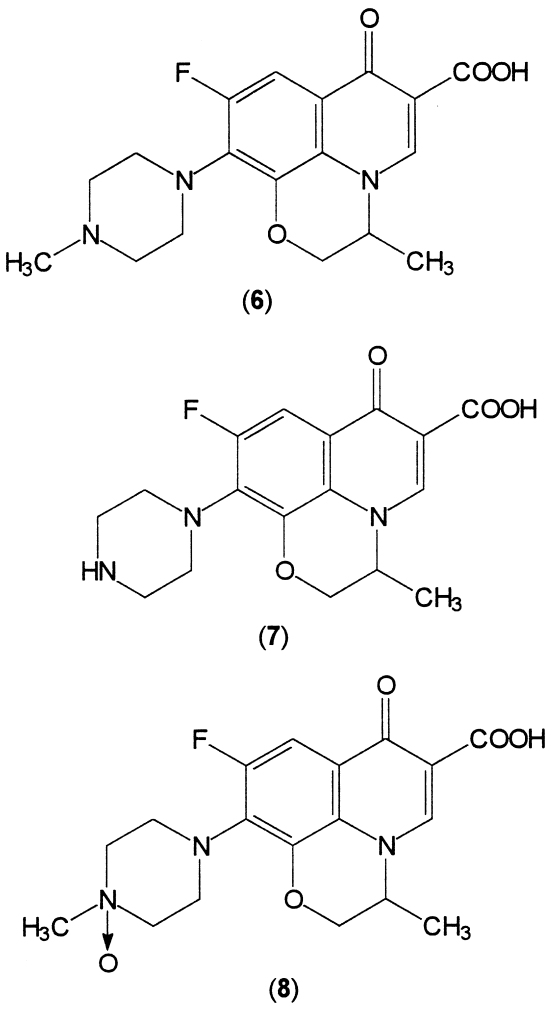


Fig. 3. Chemical structure of ofloxacin and its metabolites.

Ofloxacin (**6**) and the metabolites demethyl-ofloxacin (**7**) and ofloxacin-*N*-oxide (**8**) were determinated using a HPLC method by Borner et al. [67].

The method consists of reversed-phase chromatography and fluorimetric detection. The stationary phase was Nucleosil 5-C_{18} used at room temperature. The detector was operated under the following conditions: excitation, 295 nm; emission, 418 nm.

Detection limits of the method were 0.02 $\mu g\ ml^{-1}$ in serum and 0.2 $\mu g\ ml^{-1}$ in urine for ofloxacin (**6**) and its two metabolites. The mobile phase consisted of a mixture of 5 m*M* tetrabutylammonium phosphate–acetonitrile adjusted to pH 2.0. For improved separation of the demethylmetabolite, the pH was adjusted to 5.

Mizuno et al. [79] described a procedure for determination of ofloxacin (**6**) and other fluoroquinolones in human hair by HPLC and fluorescence detection. Briefly, samples were dissolved in 0.5 ml of 1 *M* NaOH by heating at 80°C for 30 min. The solution was neutralized with 0.1 *M* HCl and potassium hydrogen citrate buffer (pH 4.6). The internal standard was added. The mixture was cleaned up on a C_8 cartridge; the elution was effected with a mixture of tetrahydrofuran (THF)–25 m*M* orthophosphoric acid. The eluate was evaporated to dryness and the residue was reconstituted in 150 μl of mobile phase. A 60 μl portion of the solutions was analyzed on a TSKgel ODS-80Ts column (5 μm, 150 mm×4.6 mm, I.D.) with acetonitrile–25 m*M* orthophosphoric acid adjusted to pH 3 with 0.5 *M* tetrabutylammonium hydroxide as mobile phase and detection at 295 nm excitation and 490 nm emission. Several papers describe the determination of ofloxacin (**6**) in body fluids using extraction and purification steps. However, these involve tedious extraction procedures and low extraction yields.

The work of Ohkubo et al. [65] was therefore directed towards the determination of ofloxacin (**6**) in biological fluids by direct injection using column-switching techniques. A combination of a precolumn and an analytical column was selected so that interfering endogenous substances and the quinolone derivatives had different interactions with the two columns. The separation of endogenous substances and the quinolone derivatives was satisfactory using stationary phase of phenyl on the precolumn and octadecyl on the analytical column. The mobile phase consisted of 0.5% sodium acetate (pH 2.5) and acetonitrile for the two columns. The limit of detection for ofloxacin (**6**) was 20 $ng\ ml^{-1}$. Okazaki et al. [77] developed a method for the HPLC determination of (*S*)-(−)-ofloxacin and its metabolites in serum and urine using a solid-phase cleanup. While the quantification of the enantiomers of ofloxacin (**6**) in biological fluids by HPLC was proposed by Lehr et al. [73]. In these report two methods for the determination of (+)- and (−)-ofloxacin in biological fluids by HPLC are described. The first method is separation on a Resolvosil-BSA-7 (150 mm×4 mm I.D.) chiral stationary phase with bovine serum albumin immobilized on silica gel. The mobile phase was 0.2 *M* phosphate buffer (pH 8.0)–propan-2-ol. The second is the coupling of ofloxacin to L-leucinamide via diphenylphosphinyl chloride activation. Chromatographic separation of the diastereoisomeric ofloxacin (**6**) derivatives was achieved on a 125 mm×4.6 mm I.D. column filled with Nucleosil 120-5 C_{18}. The mobile phase was 0.2 *M* orthophosphoric acid adjusted to pH 1.85 with tetraethylammonium hydroxide solution, and acetonitrile (80:20). In 1988, it was reported that concomitant administration of a quinolone antibacterial agent (enoxacin) and non steroidal anti-inflammatoty agent (fenbufen) induced convulsions in several cases. In order to study a pharmacokinetic of ofloxacin (**6**), fenbufen and felbinac, an HPLC method for the simultaneous determination of this drugs, in plasma was developed by Katagiri et al. [61].

Fenbufen is known to be a pro-drug, which is metabolized to an active compound, 4-biphenylacetic acid (felbinac). Briefly, samples were analyzed by using a Chemcosorb 5ODS-H (5 μm, 150 mm×4.6 mm I.D.) column after extraction with dichloromethane–isoamyl alcohol. The mobile phase consisted of methanol–0.005 *M* sodium laurylsulphate, and adjusted to pH 2.5 with phosphoric acid. The nalidixic acid was used as an internal standard. The limits of determination of ofloxacin (**6**), fenbufen and felbinac by the present method were 0.15, 0.3 and 0.45 $\mu g\ ml^{-1}$, respectively.

4. Ciprofloxacin

Ciprofloxacin (**9**) is the most potent quinolone against Gram-negative bacteria. It is particularly active against *Pseudomonas aeruginosa*. Against

Gram-positive bacteria, ciprofloxacin is less active than some of the more recently developed quinolones [89–99,111,114,118,119,122]. Its chemical name is 1-cyclopropyl-6-fluoro-1,4-dihydro- 4-oxo-7- (1-piperazinyl)-3-quinoline carboxylic acid. The pharmacokinetics and pharmacodynamics of ciprofloxacin (**9**), after oral and intravenous administration has been reviewed by several authors [97–110, 112,113,116, 117,120,121,123, 125,127,129, 138,158, 159,161].

Ciprofloxacin (**9**) is well absorbed after oral administration. Approximately 60% of unchanged ciprofloxacin and 15% of metabolites are recovered from the urine 24 h after administration. Although ciprofloxacin (**9**) is primarily excreted by the kidneys, over 40% can be excreted in the bile if the renal route is not available.

The metabolites of ciprofloxacin (**9**) are sulfociprofloxacin (**10**), oxociprofloxacin (**11**) and desethylene ciprofloxacin (**12**) and formylciprofloxacin (**13**) are shown in Fig. 4. This was confirmed by Joos et al. [91], against Gram-positive bacteria, ciprofloxacin (**9**) is less than some of these showed a good correlation between a biological assay and HPLC for ciprofloxacin (**9**) concentrations in serum, but they obtained significant differences between these two methods with drug concentrations in urine.

The results obtained with the bioassay were markedly higher than the HPLC values, which might be due to microbiologically active metabolites excreted by the kidney. Because the metabolites are also supposed to be active in biological assays, it was necessary to develop a method suitable for distinguishing between the parent substances and their metabolites.

Several studies dealing with determination of ciprofloxacin (**9**) in blood [112,123,127,137], serum [91,98,103,105,108–110,112,120,121,124,126,127, 129,131–134,136,139–142,144–147,150,152–156], plasma [127, 135, 138, 143–145, 148, 151, 152, 157, 162], urine [91,98,108,120,123,126,131,132,134, 137, 139–141, 144–147, 150, 152, 153, 155], feces [126,145], bone [92], muscle [92], tissue [120,121, 124,130,133,142,145], bile [126,128,130,131,145, 150], saliva [103,125,134,141,150,157] showed no interference from other biological compounds.

Microbiological assay of ciprofloxacin (**9**) developed by several authors [91,97–99,101,102,104,

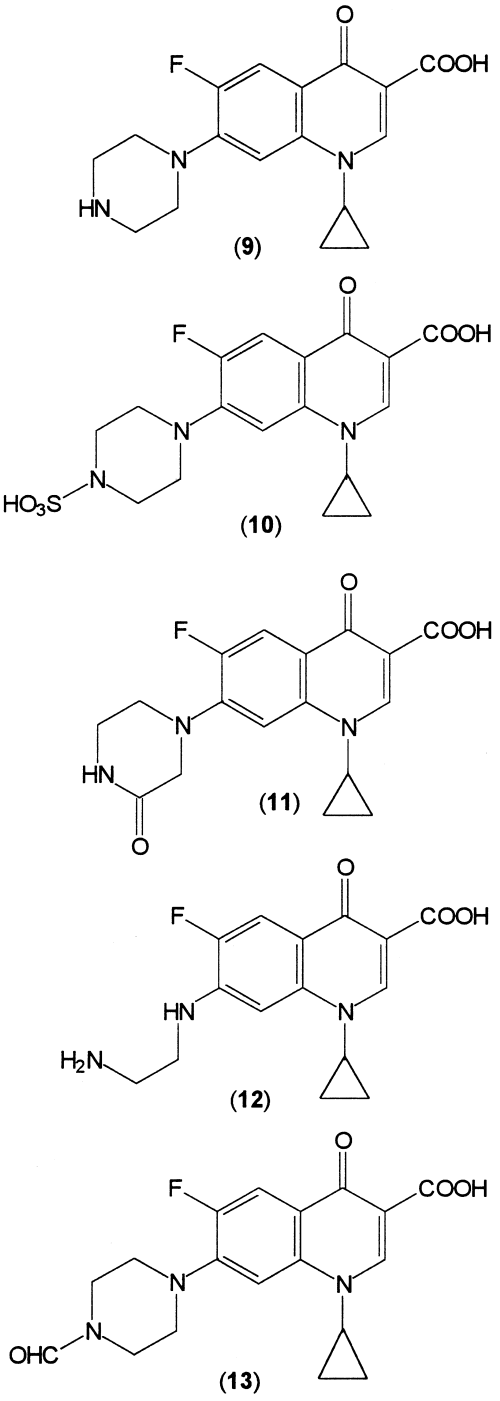


Fig. 4. Structures of ciprofloxacin and its metabolites.

106, 108, 113, 115, 117, 120, 121, 123–126, 132, 137, 154], was performed by standard procedures.

Comparison of HPLC and bioassay for determination of ciprofloxacin (**9**) in serum and urine were proposed by some authors [91,98,108,117,123,126, 132,140,147,154].

A large number of high-performance liquid chromatographic methods have been reported for the analysis of ciprofloxacin (**9**) in biological fluids [92, 98,103,105,108,110,112,116,117,123,126–157,160, 162–170].

Some of these methods have utilized UV detection [125,131–136,141,162,166,167,170], while others have utilized fluorescence detection [109, 123, 128,130,138–140,142–156,160,165].

Most of these methods have not included internal standards [91, 109, 123, 127, 128, 130, 131, 133, 136, 137,139–141,143–145,149,152,154,156,167,168].

Several methods have included the determination of ciprofloxacin metabolite concentrations [128,134, 140,141,144–146,150]. A summary of the relevant information from the analytical methods published of ciprofloxacin (**9**) is contained in Table 1.

A simple and precise HPLC procedure has been developed for the determination in biological fluids of ciprofloxacin (**9**) by Vallèe [132]. As shown in Fig. 4, free ciprofloxacin (**9**) has amphotheric properties with two ionizable groups. The pK_a values have been determined by extraction with 1,2-dichloromethane and found to be 6.0 and 8.8.

Ciprofloxacin (**9**), is one of the relatively few drugs that exhibit high magnitude of fluorescence without derivatization, which allows good sensitivity. In this procedure was used a method or quantitation of ciprofloxacin (**9**) in serum, saliva and urine samples by UV absorption. Separation of ciprofloxacin (**9**) and nalidixic acid (internal standard) was achieved using UV absorption at 313 nm, and a reversed-phase Nova–Pak C_{18} column. The mobile phase consisted of phosphate buffer adjusted to pH 7.4–methanol (65:35, v/v), and 5.5 m*M* hexandecyltrimethylammonium bromide.

Serum sample preparation involved protein precipitation with acetonitrile, followed by dichloromethane and 2-propanol extraction. After evaporation, the sample was reconstituted with a minimal volume of mobile phase. The sensitivity of the assay was 0.06 $\mu g\,ml^{-1}$. Jehl et al. [131] have developed a specific assay or the measurement of this antibiotic in human serum, bile and urine, by means of reversed-phase HPLC. The work-up procedure involves a chemical extraction step followed by isocratic chromatography on a reversed-phase Ultrasphere analytical column with ultraviolet detection at 254 nm.

The assay did not include internal standard. The detection limit for serum levels is 10 $ng\,ml^{-1}$. Briefly, an aliquot of biological fluid (serum, urine or bile) is added to dichloromethane in a glass screw-capped tube. After centrifugation the upper layer is discarded, and the organic phase is then back-extracted using phosphoric acid at pH 2.0. Reversed-phase chromatography on a C_{18} bonded silica matrix seemed to us more reproducible. Indeed, the authors carried out various assays with classical mobile phases generally used in reversed-phase HPLC, e.g. a mixture of water or salt solution with a non-polar organic solvent, and every time it resulted in severe peak tailing of ciprofloxacin (**9**), whatever the pH or the ionic strength. Finally, using ion-pairing chromatography with tetrabutylammonium bromide (TBA), an excellent resolution of ciprofloxacin (**9**) was obtained. The methodology described by Tyczkowska et al. [133] involves the determination of ciprofloxacin (**9**) in serum and prostatic tissue. Sample preparation was simplified by using ultrafiltration (rather the solvent extraction) and reversed-phase ion-paired chromatography. Detection was achieved using a photodiode array (PDA) detector. The PDA detector was used to develop the assay since it produce multiple chromatograms allowing the evaluation of chromatographic peak homogeneity.

The LC separations were performed using a mobile phase consisting of acetonitrile–methanol–water (15:2:83, v/v/v) containing 3 m*M* dodecanesulfonate, 1.5 m*M* octanesulfonate, 0.4% phosphoric acid (85%, v/v), and 0.4% (v/v) triethylamine. The column was 3 μm, 100 mm×4.6 mm phenyl Spherisorb. The limit of detection for ciprofloxacin was 2 $ng\,ml^{-1}$. Pou-Clave et al. [136], developed a simple and rapid method. A Nucleosil C_{18} reversed-phase column (30 mm×4.6 mm) was used with a mobile phase of acetonitrile–0.05 *M* KH_2PO_4 (pH 3.0)–0.1 *M* TBA. Detection was accomplished by UV absorption at 277 nm.

The total analysis time per sample is of the order

Table 1
Published HPLC analytical methods for ciprofloxacin

Application	Mobile phase	Column	Detection wavelength (nm)	Internal standard	Detection limit	Ref.
Plasma	0.1 *M* dibasic ammonium phosphate (pH 2.5)–nacetonitrile–methanol	Octadecylsilica (10 μm)-guard-pak precolum	FL 277 ex, 453 em	Yes	0.05 $\mu g\,ml^{-1}$	[148]
Serum	H_3PO_4–TBAOH (pH 3.0)–acetonitrile	μBondapak	FL 254 ex, 425 em	No	0.05 $\mu g\,ml^{-1}$	[154]
Serum, plasma, Urine	25 m*M* H_3PO_4–(pH 3.0) TBAOH–acetonitrile	Spherisorb ODS2 (5 μm)	FL 277 ex, 445 em	No	11 $ng\,ml^{-1}$	[152]
Serum, tissue	Acetonitrile–methanol–water–3 m*M* dodecanesulfonate, 1.5 m*M* octanesulfonate, 0.4%	Spherisorb	PDA, UV 278.6	No	2 $ng\,ml^{-1}$	[133]
Serum, urine	$KH_2PO_4$0–TBAOH (pH 3.0) H_3PO_4–acetonitrile–methanol	μBondapak C_{18} (5 μm)	FL 278 ex, 470 em	Yes	1 $ng\,ml^{-1}$	[146]
Plasma	30 m*M* phosphate buffer (pH 3.0)–20 m*M* TEA–20 m*M* SDS–acetonitrile	Keystone ODS (5 μm)	FL 278 ex, 470 em	Yes	5 $ng\,ml^{-1}$	[151]
Serum, urine, sputum	Acetonitrile–0.1 *M* potassium phosphate buffer (pH 2.5) H_3PO_4–KOH	Micro Pak MCH 10 (10 μm), precolumn Vydac (40 μm)	UV 280 FL 330 ex, 440 em	No	0.02 $\mu g\,ml^{-1}$	[141]
Serum, plasma, bile, faeces, tissue	TBA–bisulfate–acetonitrile	Nucleosil 120-5 C_{18} (5 μm)	FL 278 ex, 445 em	No	–	[145]
Serum, urine	Acetonitrile–25 m*M* H_3PO_4 (pH 3.0)–TBAOH	Nucleosil C_{18} (5 μm)	FL 278 ex, 445 em	No	0.01 $\mu g\,ml^{-1}$	[139]
Serum, urine, bile	Acetonitrile–50 m*M* TBABr–H_3PO_4 (pH 2.0)	Ultrasphere C_{18} (5 μm)	UV 254	No	10 $ng\,ml^{-1}$	[131]
Serum, urine	Phosphate buffer (pH 3.0)–methanol–acetonitrile	μBondapak C_{18} (10 μm)	FL 270 ex, 440 em	Yes	0.08 $\mu g\,ml^{-1}$	[153]
Serum, saliva	Methanol–phosphate buffer (pH 7.4)–5.5 m*M* HDTA Br–NaOH	Nova–Pak Radial Pak	UV 313	Yes	0.06 $\mu g\,ml^{-1}$	[132]
Plasma, saliva	Methanol–80 m*M* KH_2PO_4 (pH 2.5)–H_3PO_4, 6 m*M* TBAOH	μBondpak C_{18} (10 ≅m)	UV 268	Yes	0.05 $\mu m\,ml^{-1}$	[157]
Plasma	Acetonitrile–methanol–0.1 *M* citric acid–ammonium perchlorate–TBAOH	μBondapak C_{18} (10 μm)	FL 270 ex, 440 em	Yes	0.1 $\mu g\,ml^{-1}$	[138]
Serum, urine, saliva	0.1 *M* KH_2PO_4 (pH 2.6)–H_3PO_4 (A)–KH_2PO_4–acetonitrile (B)	Spheri-5 ODS-5A (5μm)	UV 280	Yes	0.05 $\mu g\,ml^{-1}$	[134]
Serum, plasma, urine	Acetonitrile–25 m*M* H_3PO_4 (pH 3.0)–TBAOH	Spherisorb ODS-2 (5 μm)	FL 277 ex, 445 em	No	0.01 $\mu g\,ml^{-1}$ 0.05 $\mu g\,ml^{-1}$	[123]
Urine	KH_2PO_4 (pH 3.0)–H_3PO_4 (A)–acetonitrile (B)	Spherisorb ODS-2 (5 μm)	UV 278			[170]
Bile	H_3PO_4–TBAOH (A) acetonitrile (B)	μBondapak C_{18} (10 μm)	FL 278 ex, 445 em	No	0.5 $\mu g\,ml^{-1}$	[128]
Plasma, urine	Phosphate buffer–acetonitrile–TBAOH	Nova–Pak C_{18} (4 μm)	FL 278 ex, 445 em	No		[168]
Serum	50 m*M* KH_2PO_4 (pH 3.0)–acetonitrile–0.1 *M* TBABr	Nucleosil C_{18} (3 μm)	UV 277	Yes	0.1 $\mu g\,ml^{-1}$	[136]
Serum, plasma, urine	20 m*M* TCA–acetonitrile–methanol (pH 3.0)	PRLP-S	FL 277 ex, 418 em	No	0.05 $\mu g\,ml^{-1}$	[144]
Humor aqueous	Acetonitrile–methanol–0.4 *M* citric acid	Nova–Pak C_{18} (4 μm)	FL 278 ex, 445 em	No	250 $pg\,ml^{-1}$	[149]
Plasma	Methanol–0.2 *M* ammonium acetate (pH 2.7)	Shimpack CLC-DS (5 μm)	UV 280	No	52 $ng\,ml^{-1}$	[143]

(Continued overleaf)

Table 1. Continued

Application	Mobile phase	Column	Detection wavelength (nm)	Internal standard	Detection limit	Ref.
Serum, urine	25 m*M* H_3PO_4 (pH 3.0)–TBAOH–acetonitrile	Spherisorb ODS-2 (5 μm)	FL 277 ex, 456 em	No	0.01 μg ml^{-1}	[137]
Blood, serum, urine	25 m*M* H_3PO_4 (pH 3.0)–TBAOH–acetonitrile	Spherisorb ODS-2 (5μm)	FL 278 ex, 456 em	No	0.03 μg ml^{-1}	[91]
Serum, bile, saliva, urine	Acetonitrine–methanol–50 m*M* KH_2PO_4–H_3PO_4 (pH 3.0)–0.1 *M* TBABr	Nucleosil C_{18} (5μm)	FL 277 ex, 360 em	Yes	0.01 μg ml^{-1}	[150]
Serum	Methanol–67 m*M* phosphate buffer (pH 3.5)	μBondapak C_{18} (10 μm)	FL 277 ex, 445 em	No	0.005 μg ml^{-1}	[156]
Serum, urine	5 m*M* TBA–phosphate (pH 2.0)–H_3PO_4–accetonitrile	Nucleosil 5-C_{18} (5 μm)	FL 275 ex, 418 em	No	10 μg l^{-1} 200 μg l^{-1}	[140]
Serum, urine	Acetonitrile–TBAOH–phosphate buffer (pH 3.0) H_3PO_4	μBondapak C_{18} (10 μm)	FL 278 ex, 456 em	Yes	0.15 μg ml^{-1}	[147]
Serum, urine	H_3PO_4 (pH 3.0)–TBAOH–methanol	Brownlee C_{18} (5 μm)	FL 278 ex, 447 em	No	20 ng ml^{-1}	[109]
Serum, urine, saliva, blister fluid	Phosphate buffer–methanol–5.5 m*M* HDTA (pH 7.4)	Nova–Pak C_{18} (4 μm)	UV 313	Yes	0.06 μg ml^{-1}	[125]
Serum, urine	2 m*M* TBAOH (pH 2.5)–H_3PO_4–methanol–acetonitrile	μBondapak C_{18} (10 μm)	UV 254	Yes	0.25 μg ml^{-1}	[166]
Serum, urine	Methanol–THF–0.67 *M* phosphate buffer (pH 3.0)	μBondapak C_{18} (10 μm)	DAD	No	0.01 μg ml^{-1}	[155]
Blood, serum, urine	Acetonitrile–25 m*M* phosphate buffer (pH 3.0)–TBAOH	Versapak C_{18} (10 μm)	FL 274 ex, 418 em	Yes	0.008 μg ml^{-1}	[169]
Plasma, urine, saliva	Methanol–THF–67 m*M* (pH 3.0)	Radial-Pak C_{18} (10 μm)	FL 277 ex, 445 em	No	5 ng ml^{-1}	[127]
Plasma	Methanol–acetonitrile–0.4 *M* citric acid	μBondapak C_{18} (4 μm)	UV 275 nm	Yes	0.05 μg ml^{-1}	[135]
Blood	Acetonitrile–100 m*M* NaH_2PO_4–(pH 3.9) H_3PO_4	μBondapak C_{18} (10 μm)	FL 280 ex, 455 em	Yes	25 ng ml^{-1}	[165]
Perfusate, bile	Acetonitrile–25 m*M* phosphate buffer–5 m*M* TBABr	Lichrosorb RP-18 (5μm)	FL 270ex, 440 em	No	100 ng ml^{-1}	[130]
Blood	Methanol–5 m*M* laurylsulfate (pH 2.5)–H_3PO_4	Chemcosorb 5 ODS-H	UV 275	Yes	200 ng ml^{-1}	[162]
Urine	Acetonitrile–50 m*M* citric acid–1 *M* ammonium acetate	Nucleosil C_{18}	FL 280ex, 418 em	Yes	2 μg ml^{-1}	[164]
Brain	Methanol–5 m*M* laurylsulfate (pH 2.5)–H_3PO_4	Wacosil 5 C_{18}	FL 277ex, 445 em	Yes	10 ng g^{-1}	[142]

TBAOH=Tetrabutylammnium hydroxide; TBABr=tetrabutylammonium bromide; HDTA=hexadecyltrimethylammonium; FL=fluoresccence detection; ex=excitation wavelength; em=emission wavelength.

of 5–7 min. Mehta et al. [135], use 5 ml dichloromethane (containing pipemid acid as internal standard) to extract 0.5 ml of plasma. The organic phase containing ciprofloxacin (**9**) was then back-extracted into the acidic 0.5 *M* phosphoric acid at pH 2.0. The wavelength of the UV detector was set at 275 nm.

Gladys Mack [134] utilized UV absorbance detection at 280 nm for the measurement of ciprofloxacin (**9**) and its metabolites in human specimens. The sensitivity limits for ciprofloxacin, desethylene (**12**)-ciprofloxacin (**9**), sulfociprofloxacin (**10**) and oxociprofloxacin (**11**) were 0.03, 0.25, 0.25 and 0.25 μg ml^{-1}, respectively. Tinidazole is used as an internal standard. Separation of ciprofloxacin (**9**) and its metabolites was achieved at room temperature using a gradient system.

A HPLC assay has been reported to measure simultaneous ciprofloxacin (**9**) and theophylline in

plasma or saliva samples by Zhai et al. [157]. This assay can be used to investigate drug interactions involving theophylline and this fluoroquinolone.

Briefly, the biological samples were extracted with dichloromethane–isopropyl alcohol prior to isocratic chromatography on a μBondapak C_{18} column. Ultraviolet detection was carried out at 268 nm. The assay is linear for ciprofloxacin (**9**) (0.05–10 $\mu g\,ml^{-1}$), and theophylline (0.1–20 $\mu g\,ml^{-1}$). The detection limit was 0.05 $\mu g\,ml^{-1}$ for ciprofloxacin (**9**), and 0.1 $\mu g\,ml^{-1}$ for theophylline. No interference by other methylxantines was noted. Difloxacin was used as the internal standard.

An assay system based on direct injection of serum and urine samples was developed by Nilsson-Ehle [139].

Serum samples were injected without any pretreatment except addition of an equal volume of distilled water which facilitated the passage through 0.6 μm Millipore filters. Urine samples were diluted depending on the expected concentration of the analite.

An internal standard is therefore not needed, as shown also by the excellent precision of the assay. The sensitivity of the assay allows determination of concentrations as low as 0.01 $\mu g\,ml^{-1}$. A fluorimetric detector with an excitation 278 and emission at 445 nm is used. No interference from concurrent administration of furosemide, paracetamol, prednisolone, warfarin, digoxin and other drugs was noted.

A versatile and sensitive HPLC assay has been developed by Shah et al. [138]. Measurements of plasma ciprofloxacin (**9**) concentrations were determined by HPLC method with fluorescence detection. Briefly, after precipitation of the plasma proteins with a mixture of acetonitrile and perchloric acid, the extract was injected onto a μBondapak C_{18} column preceded by a C_{18} guard column.

The mobile phase consisted of acetonitrile–methanol, and 0.1 *M* citric acid containing 0.54 g ammonium perchlorate and 0.65 ml of tetrabutylammonium hydroxide. A fluorescence detector with an excitation wavelength of 270 nm and an emission wavelength of 440 nm was used. Abbott compound 56619 was used as an internal standard. The assay was linear over the range of 0.05 to 10 $\mu g\,ml^{-1}$. Other HPLC assays have been reported.

The separation and isolation of ciprofloxacin (**9**) from human serum utilizing reversed-phase HPLC was accomplished by Fashing et al. [154]. The analysis was carried out using a μBondapak C_{18} column and a mobile phase mixture consisting of acetonitrile–25 m*M* phosphoric acid and 30 m*M* tetrabutylammonium hydroxide at pH 3.0. Fluorescence detection was achieved using a 254 nm wavelength excitation and a 425 nm wavelength emission. Calculations were based on an external standard method using peak height ratios. The limit of detection was 0.05 $\mu g\,ml^{-1}$.

An HPLC method for the determination of ciprofloxacin (**9**) concentrations in biological fluids has been reported by Gau et al. [152] with claims of simplicity and rapidity of sample treatment. Indeed, diluted serum, plasma and urine samples are injected onto a Spherisorb ODS II column without prior extraction or clean-up procedure. Ciprofloxacin (**9**) was separated from other compounds by an eluent consisting of an 0.025 *M* phosphoric acid solution adjusted to pH 3.0 with tetrabutylammonium hydroxide and acetonitrile. The limit of detection were 11, 11 and 50 $ng\,ml^{-1}$ for plasma, serum and urine, respectively.

Nix et al. [153] developed an HPLC assay for the detection of ciprofloxacin (**9**) in serum and urine. Serum samples were prepared by precipitating protein with perchloric acid. Urine samples were diluted 100-fold with mobile phase. The mobile phase consisting of pH 3.0 phosphate buffer–acetonitrile–methanol. Separations were carried out on a μBondapak C_{18} reversed-phase column. With the use of the internal standard, A-56619, quantitation was obtained to less than 0.08 $\mu g\,ml^{-1}$ for serum.

A rapid HPLC assay has been developed by Lovdahl et al. [151] for the detection of ciprofloxacin (**9**) in chinchilla middle ear and plasma. Ciprofloxacin (**9**) and the internal standard, difloxacin, were separated on a Hypersil ODS column (100 mm×2.1 mm I.D., 5μm) using a mobile phase of 30 m*M* phosphate buffer (pH 3.0), 20 m*M* triethylamine, 20 m*M* sodium dodecylsulphate and acetonitrile. Fluorimetric detection of ciprofloxacin (**9**) and the internal standard employed excitation at 278 nm and emission at 456 nm. The detection limit for ciprofloxacin (**9**) was 5 $ng\,ml^{-1}$.

Ciprofloxacin (**9**) concentration was measured in serum and urine by HPLC by Drusano et al. [167].

The serum assay used protein precipitation, reversed-phase HPLC, and fluorimetric detection.

The urine assay used sample dilution followed by direct injection. Quinine was used as an internal standard. A C_{18} μBondapak reversed-phase column was used for the assay. The mobile phase consisted of methanol–tetrahydrofuran, and phosphate buffer (pH 3.0). The column was heated to 50°C. The sensitivities of the assays were at least 0.01 μg ml^{-1}.

A rapid liquid chromatographic assay of ciprofloxacin (**9**) in human aqueous humor after direct injection was described by Basci et al. [149]. Briefly, to a glass-capped tube, 50 μl of aqueous humor sample, 350 μl of distilled water and pipemidic acid as an internal standard were added. 20 μl of solution were used for HPLC analysis.

The separation was performed on a Nova–Pak C_{18} cartridge (100 mm×8 mm I.D., particle size 4 μm) compressed in a Radial–Pak holder in conjunction with a precolumn module containing a Nova–Pak C_{18} insert. The mobile phase consisted of methanol–acetonitrile–0.4 *M* citric acid. Fluorescent detector excitation wavelength was set at 278 nm and emission wavelength at 450 nm, respectively.

The detection limit was 250 pg ml^{-1} for ciprofloxacin (**9**). An one-step extraction procedures are used for the determination of ciprofloxacin (**9**) and 7-ethylenediamine metabolite in human serum and urine by Awni et al. [146]. Samples were analyzed by using a C_{18} μBondapak reversed-phase column after extraction at pH 7.4 with dichloromethane. After evaporation of the organic layer, samples were reconstituted with mobile phase.

The mobile phase consisted of methanol, acetonitrile, tetrabutylammonium hydroxide in 0.02 *M* potassium dihydrogenphosphate, adjusted with phosphoric acid to pH 3.0. Difloxacin was used as an internal standard. Fluorescence detection was achieved using a 278 nm wavelength excitation and a 420 nm wavelength emission. Ciprofloxacin (**9**) concentration as low as 1 ng ml^{-1} in serum of urine, were easily measurable using the method described.

A sensitive and selective determination of picogram amounts of ciprofloxacin (**9**) and its metabolites (**10**, **11**, **12**, and **13**) in urine, serum, plasma, bile, faeces and tissue using HPLC and photothermal post-column derivatization was developed by Scholl et al. [145].

The assay consists of reversed-phase HPLC and fluorescence detection. For the liquid matrices the only sample preparation that is required is dilution. For the metabolites (**10**, **11** and **13**) an additional post-column derivatization by successive thermolysis and photolysis is needed. The mobile phase consisted of a mixture of acetonitrile and tetrabutylammonium bisulfate. The external standard method was employed.

Separation were carried out on Nucleosil 120-5 C_{18}, 5 μm (250 mm×4 mm I.D.). Detection limits for all compounds are between 0.2 and 2.2 ng ml^{-1}. Krol et al. [144] developed an improved HPLC assay for the detection of ciprofloxacin (**9**) and ciprofloxacin metabolites in body fluids.

The previously published HPLC procedure by the same authors described the isocratic separation of ciprofloxacin (**9**) and three ciprofloxacin metabolites in urine sample on a polystyrene–divinylbenzene column followed by quantitation using a UV detection. The present procedure involved the same chromatographic separation, but it was also applicable to the analysis of plasma and serum as well as urine samples, and quantitation was based on fluorimetric detection after post-column induction of fluorescence instead of UV detection.

The post-column induction of fluorescence was necessary because the (**10**) and (**11**) metabolites of ciprofloxacin (**9**) have relatively weak native fluorescence, and induction enhanced the fluorimetric signals of metabolites (**10**) and (**11**) forty-four-fold and eleven-fold, respectively. The lower quantitation limits of ciprofloxacin (**9**) and metabolites were 0.05 μg ml^{-1}, 0.01 μg ml^{-1}, 0.05 μg ml^{-1}, and 0.5 μg ml^{-1}, respectively.

5. Pefloxacin

Pefloxacin (**14**) (1589 RB, EU-5306) which has the formula 1-ethyl-6-fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (**14**) (Fig. 5) is a piperazinyl substituted quinolone derivative being developed by Rhone–Poulenc for oral and parenteral use.

In man, pefloxacin is metabolized into *N*-demethylpefloxacin (**2**) and *N*-oxide derivatives (**15**)

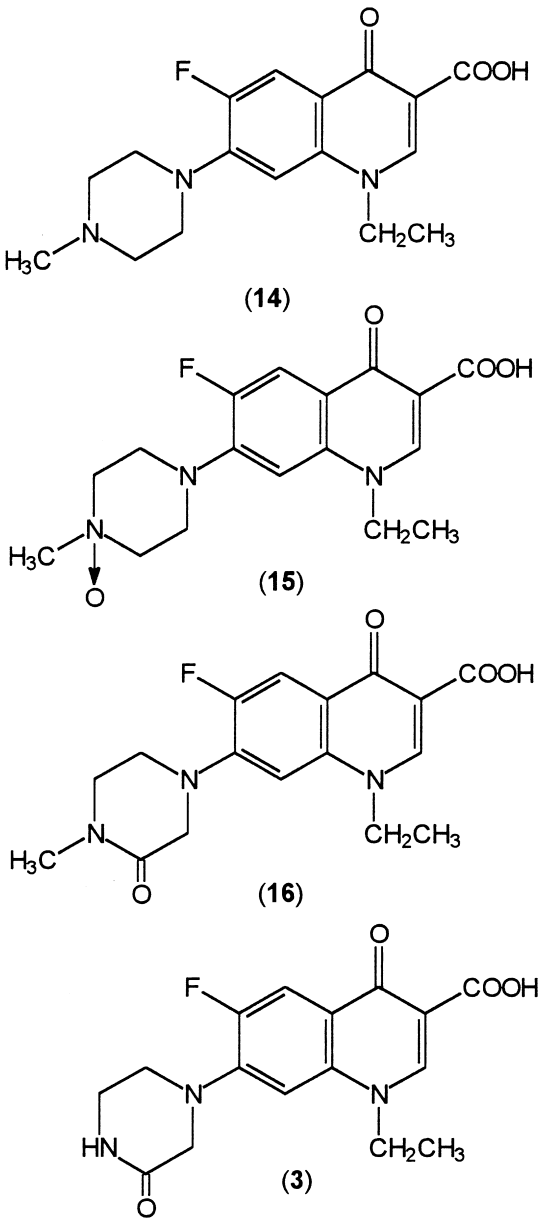


Fig. 5. Chemical structure of pefloxacin and its metabolites.

and also into oxopefloxacin (**16**) and oxonorfloxacin (**17**), pefloxacine glycuronide is not found in human bile or urine [171].

Urine is the major route of elimination in man and unchanged compound and norfloxacin, pefloxacine *N*-oxide (**15**), oxonorfloxacin (**17**), and oxopefloxacin (**16**) represent 58.9% of the oral dose. The *N*-demethyl derivative (**2**) presents the same in-vitro activity as pefloxacin whereas the *N*-oxide derivative not have antibacterial activity.

Lacarelle et al. [172] developed an enzyme-linked immunosorbent assay (ELISA) that permits direct determination of pefloxacin in human serum and validated this new assay by comparing results measured by high-performance liquid chromatography with those obtained with this new immunoassay technique.

Few methods are available for the quantification of pefloxacin in biological fluids. These include microbiological assays [173] and HPLC assays [174–177]. Some of these methods are time-consuming and involve tedious extraction steps.

Concentrations of pefloxacin (**14**), norfloxacin (**2**) and pefloxacin *N*-oxide (**15**) were analyzed by HPLC with spectrofluorimetric detection by Montay et al. [172].

This reversed-phase HPLC method was reported for quantitating pefloxacin and its main active metabolites in plasma and urine using UV absorption at 270 nm. In this author's experience, the method has also been applied to plasma and urine from humans with good results. Chromatography equipment was a Varian Model 5020 equipped with a Pye–Unicam LC–UV detector.

A reversed-phase LiChrosob RP-18 column was used. The mobile phase consisted of a mixture of water and acetonitrile containing 0.4% of Na_2HPO_4 and 0.2% of tetrabutylammonium iodide. Adjusted to pH 9.4 with 1% TEA. Two solvents were prepared: solvent A was only distilled water; solvent B was an acetonitrile–water (2:3, v/v) mixture. The mobile phase was 52% of solvent A plus 48% of solvent B for pefloxacin assay; for pefloxacin, norfloxacin and oxonorfloxacin assay a gradient of B was used starting from 20% of B and rising at rate of 2.5% per min for 10 min. The reversed-phase HPLC method of Xu et al. [178] utilizes a simple and efficient methanol extraction of heparin-treated plasma. No internal standard is used.

Other authors reported an HPLC method for various fluorquinolones, however, the resolution between pefloxacin and its metabolites was lacking [176]. Abanmi et al. [179] described a simple, rapid and isocratic HPLC assay for the determination of pefloxacin and its main active metabolite, norflox-

acin, in serum. Briefly, this method involves protein precipitation with acetonitrile and use of the fluorescent properties of pefloxacin and its metabolites, which enhanced the sensitivity limits significantly, to 50 ng ml^{-1}, for both compounds.

Samples were analyzed by using a Nova–Pak C_{18} cartridge (Radial–Pak; 100 mm×8 mm, average particle size 4 μm). The mobile phase consisted of a mixture of acetonitrile in buffer solution. The acebutolol was used as an internal standard. The effluent was monitored at excitation and emission wavelengths of 330 and 440 nm, respectively.

In 1986, it was reported that several patients had severe chronic convulsions during therapy with quinolones and fenbufen and it is reasonable to assume that, simultaneously administered, fenbufen enhanced the neurotoxic potency of quinolones via some pharmacodynamic interaction in the brain [180].

The interest in this group of drugs has prompted us to develop a simple and sensitive assay method for both these substances in human plasma, which could be applied to pharmacokinetic studies by Carlucci et al. [181].

6. Amifloxacin

Amifloxacin (**17**) (WIN 49375) is a synthetic antibacterial agent of the quinolone class [182]. It is similar in chemical structure to pefloxacin but differs by containing a methylamino, rather than an ethyl, substituent at the 1-*N* position.

Its chemical name is 6-fluoro-1,4-dihydro-1-(methylamino)-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid. Its chemical structure is shown in Fig. 6.

Amifloxacin (**17**) is less active against Gram-positive cocci than against Gram-negative bacteria [183]. Amifloxacin (**17**) was generally less active than cefotaxime but more active than gentamicin. WIN 49375, the major piperazinyl-*N*-desmethyl metabolite (**18**) of amifloxacin (**17**), was as effective as the parent drug against experimental infections in mice when given parenterally. When administered orally, however, this metabolite was less potent than amifloxacin (**17**) [184].

Amifloxacin (**17**) was highly active by the oral

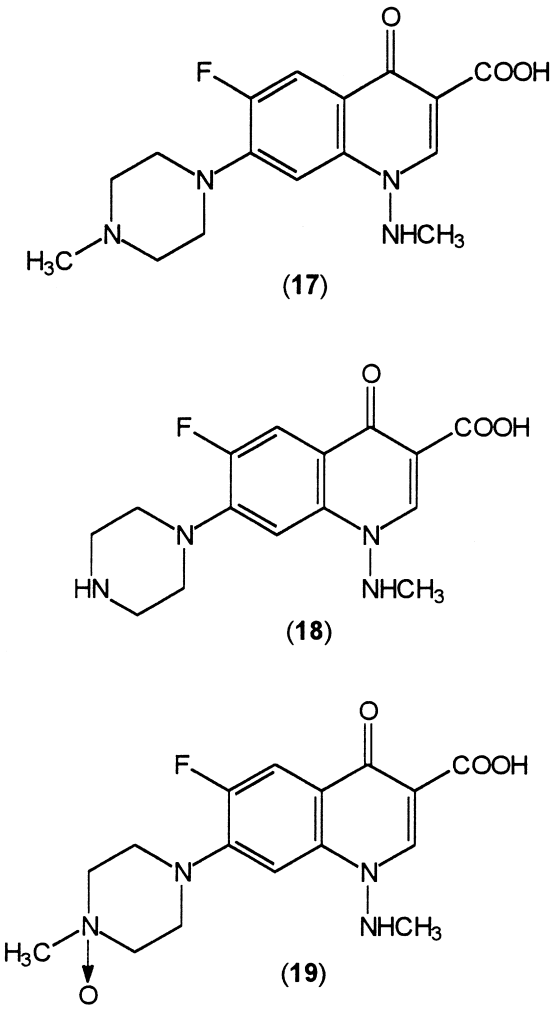


Fig. 6. Structures of amifloxacin and its metabolites.

route, with 50% effective doses within two- to threefold of those obtained with parenteral medication [185]. An analytical method by HPLC for the quantitation of amifloxacin in plasma and urine has been developed by McCoy et al. [186]. Briefly, samples were analyzed by using a μBondapak phenyl Corasil precolumn and a μBondapak phenyl Corasil column.

The method involves extraction with chloroform, back-extraction into 0.1 *M* sodium hydroxide, and subsequent analysis with UV detection at 280 nm. The rosoxacin was used as an internal standard. The mobile phase consisted of a mixture of 0.05 *M*

sodium chloride and 3 m*M* tetrabutyl ammonium phosphate adjusted to pH 2.3 with phosphoric acid and acetonitrile. The limit of detection where 0.032 $\mu g\,ml^{-1}$ for plasma and 2.7 $\mu g\,ml^{-1}$ for urine, respectively.

The automated determination of amifloxacin (**17**) and two of its principal metabolites in human plasma and urine by column-switching HPLC is described by Crawmer et al. [187]. Plasma or urine samples, diluted 1:1 with 0.5 *M* sodium citrate buffer pH 2.5, were directly injected onto a Bondapak CX Corasil, 37–50 μm particle size, 50 mm×2 mm I.D., cation-exchange pre-column. Following a 2.0 min wash of the pre-column with a flow-rate of 1.1 $ml\,min^{-1}$, the effluent from the pre-column was directed to the analytical column (Nova–Pak C_{18} 4 μm Radial–Pak cartridge) by a column switching device.

The pre-column for the analytical column consisted of Bondapak C_{18} Corasil (37–50 μm particle size, 23 mm×3.9 mm I.D.). Linear response were observed in the range 0.10–5.0 $\mu g\,ml^{-1}$ for plasma and 0.50–100 $\mu g\,ml^{-1}$ for urine for all three compounds. The minimum quantifiable levels were 0.10 and 0.50 $\mu g\,ml^{-1}$ for piperazinyl-*N*-demethyl (**18**) and piperazinyl-*N*-oxide (**19**) metabolites in plasma and urine samples obtained from humans.

7. Fleroxacin

Fleroxacin (**20**) (AM-833; Ro 23-6240), a trifluorinated quinolone, has a potent and broad in vitro activity against Gram-positive and Gram-negative bacteria and also exhibits significant in vivo activity against various experimental infections after oral administration [188–190].

Pharmacokinetic studies in healthy volunteers revealed that fleroxacin (**20**) is metabolized to a small extend and is characterized pharmacokinetically by a long elimination half-life and high concentration in plasma [191–193]. This drug was principally excreted into urine by humans [191–194].

The chemical name of fleroxacin (**20**) is 6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid. Structures of fleroxacin (**20**) and its metabolites are shows in Fig. 7. Demethylation (**21**) and *N*-oxidation (**22**) have been shown to be the main metabolic pathways in humans [190,191].

Several HPLC methods have been reported for the analysis of fleroxacin (**20**) and its main metabolite in biological fluids [194–201]. Of the few methods published for fleroxacin assay [193,194,201], one requires a gradient elution and takes about 35 min per run [201], another involves a high flow-rate (3 $ml\,min^{-1}$), which is likely to shorten column life [193].

All three have detection limits of ca. 100 $\mu g\,l^{-1}$. Brunt et al. [199] use fluorescence detection for the quantitation of fleroxacin (**20**) in erythrocyte. The purpose of this study was to investigate the red blood

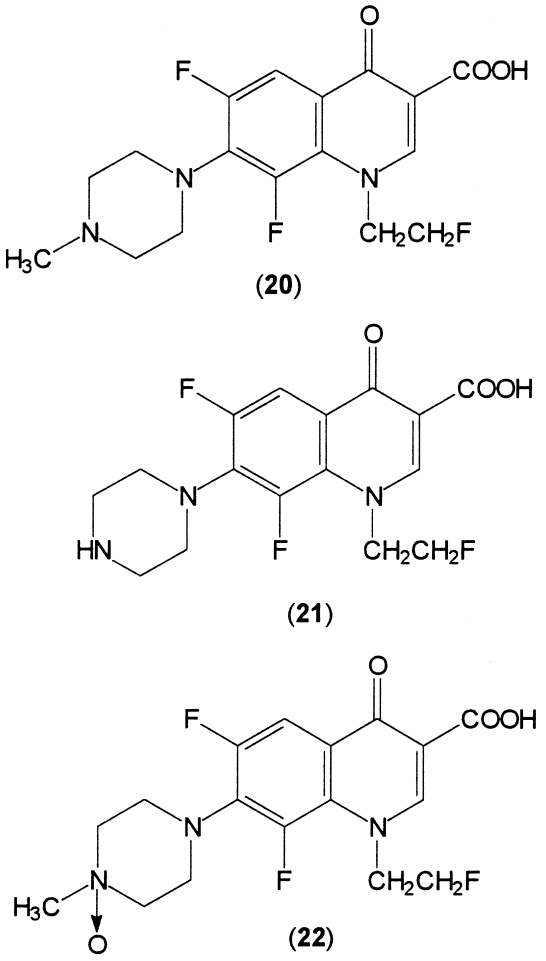


Fig. 7. Structures of fleroxacin and its metabolites.

cell partition coefficient of fleroxacin (**20**) and to compare that of another fluroquinolones.

The fluorescence response at 470 nm (281 nm excitation) is linear for fleroxacin (**20**) concentrations up to 0.2 $\mu g\,ml^{-1}$, and the sensitivity of the method is 0.1 $\mu g\,ml^{-1}$.

Awni et al. [195] use pipemidic acid as an internal standard. Sample cleanup prior to chromatographic analysis is accomplished by extraction the drug from basic-buffered serum with a mixture of ethyl acetate–isopropanol. Fluorimetric detection of fleroxacin (**20**) employed excitation at 290 nm and fluorescence at 470 nm. Standard curves were linear from 20 to at least 10000 $\mu g\,l^{-1}$ for serum and urine. The detection limit of the method was estimated to be 5 $\mu g\,l^{-1}$ for both serum and urine. No assay interferences have been observed with other drug with the analysis for fleroxacin (**20**) in human serum or urine.

A quantitative reversed-phase HPLC method for the determination of fleroxacin (**20**) and its metabolites in plasma and urine is described by Dell et al. [196]. Fleroxacin (**20**) was metabolized, and the assay was capable of measuring levels as low as 10–20 $ng\,ml^{-1}$ of the metabolites using a fluorimetric detector after separation on an octadecyl reversed-phase column with a flow-rate of 0.8 $ml\,min^{-1}$.

The fluorescence detector was operated at excitation and emission wavelengths of 290 nm and 450 nm, respectively. The mobile phase consisted of 5 m*M* tetrabutylammonium hydrogensulfate and methanol. Pipemid acid an analog of fleroxacin (**20**), is used as internal standard.

The method for simultaneous determination of fleroxacin (**20**) and its *N*-demethyl (**21**) and *N*-oxide (**22**) metabolites in plasma and urine by HPLC has been reported by Heizmann et al. [200].

Briefly, sample cleanup prior to chromatographic analysis is accomplished by extraction of the drugs with acetonitrile. The supernatant was evaporated under nitrogen. The dry residue was reconstituted in the mobile phase. The quinolone derivative of fleroxacin (**20**) was used as an internal standard for plasma samples.

Fluorimetric detection of fleroxacin (**20**), *N*-demethyl (**21**) and *N*-oxide (**22**) metabolites employed excitation at 290 nm and fluorescence at 450 nm. Samples were analyzed by using a reversed-phase TSK ODS 120T, 5 μm (250 mm×4.6 mm I.D.) connected to a precolumn.

The internal standard used for the plasma assay could not be used for the urine assay owing to the presence of an endogenous peak in some patients' urines. Pipemidic acid was therefore used for the urine assay. The mobile phase consisted of a mixture of 10 m*M* hydrogensufate–50 m*M* potassium dihydrogenphosphate–methanol. The pH of the final eluent was brought to 2.6 with 40% phosphoric acid. The limit of detection was ca. 1 $ng\,ml^{-1}$ for fleroxacin (**20**), and 10 $ng\,ml^{-1}$ for both the metabolites.

8. Difloxacin

Difloxacin (**23**) (A-56619) is a new aryl-fluoroquinolone synthesized by Chu et al. [202]. Its chemical name is 6-fluoro-1-(4-fluorophenyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (Fig. 8).

Difloxacin (**23**) is 2–4-fold less active than norfloxacin (**2**) against enteric bacteria and *Pseudomonas aeruginosa* [203,204] and 2-fold more active than norfloxacin against Gram-positive cocci. Difloxacin (**23**) has exceptional efficacy in experimental infections [205,206]. Difloxacin (**23**) was also notable in its efficacy against intracellular pathogens in experimental infections such as *L. pneumophila* and *Salmonella typhimurium* [207].

In animals, difloxacin (**23**) was well absorbed after oral administration and was extensively metabolized, primarily by glucuronidation and by oxidation to the *N*-oxide (**25**) and *N*-demethyl (**24**) analogues [208]. Bioassays have been developed for difloxacin (**23**), however, they are considered inferior to HPLC procedures, particularly when precision, sensitivity, and specificity are of primary importance (e.g. pharmacokinetic or metabolic studies).

By using HPLC, the metabolism and pharmacokinetics of difloxacin (**23**) were characterized in humans after single oral doses of 200, 400 and 600 mg by Granneman et al. [209,210]. The samples were assayed by HPLC with an Adsorbosphere HS C_{18} analytical column (250×4.6 mm I.D., 7 μm particle size).

Fluorimetric detection of difloxacin (**23**) and its metabolites employed excitation at 280 nm and

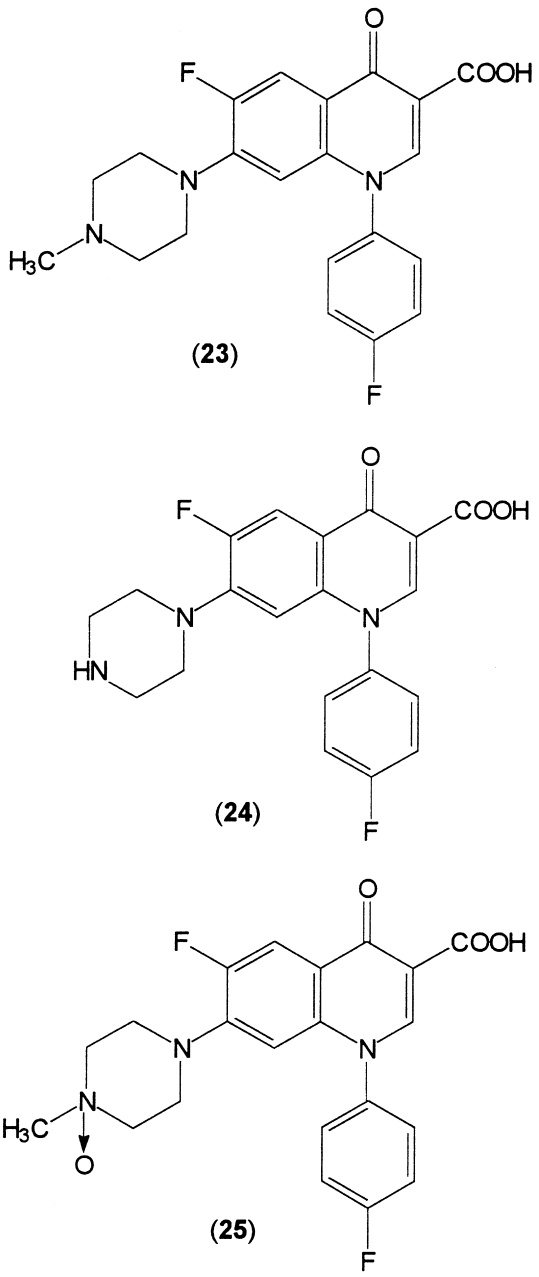


Fig. 8. Chemical structures of difloxacin and its metabolites.

emission at 389 nm. For plasma analysis, the mobile phase (pH 2.7) contained 0.05 *M* phosphate, 0.2% sodium dodecyl sulfate, and 50% acetonitrile. The quinolone derivative of difloxacin was used as an internal standard. The mixture were ultrafiltered with an Amicon Centrifree apparatus before chromatography.

For analysis of urine, the phosphate concentration of the mobile phase was increased to approximately 0.08 *M*, and the pH was increased to 5.3. With this procedure, recoveries of difloxacin (**23**) was greater than 98%. The limit of quantitation was 10 ng ml^{-1}.

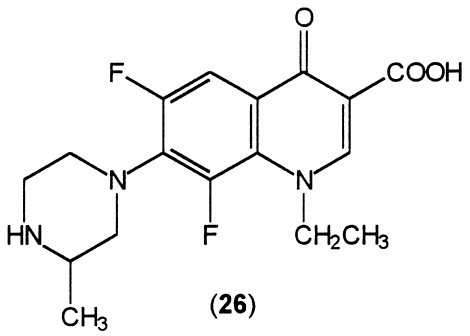
Ping et al. [211] developed a method for the determination of difloxacin (**23**) in plasma using a HPLC column-switching technique. Briefly, an automatic cleanup and concentration method by column switching is described for assay of difloxacin in plasma. The system uses YWG-C_{18} short precolumn for on-line cleanup and concentration and a Shimpack CLC-ODS analytical column for separation. The mobile phase consisted of 0.2% acetic acid is used for pretreatment, while a mixture of 0.2 *M* ammonium acetate–methanol was used for analytical mobile phase. The plasma samples were treated with trichloroacetic acid and ultrasonicated prior to inject in the precolumn. The quantitation is achieved by monitoring the UV absorbance of the eluate at 280 nm. The lower limit of detection is 192 ng ml^{-1}.

9. Lomefloxacin

Lomefloxacin (**26**) (NY-198; SC-47111) is a difluorinated quinolone antimicrobial agent with a piperazinyl group on the quinolone ring which has been demonstrated to have a broad antibacterial spectrum in vitro and in vivo, with activity similar to that of other fluoroquinolones [212,213]. The in vitro activity of lomefloxacin (**26**) is equal to or 2-fold less than that of ofloxacin (**6**) and temafloxacin against Gram- positive bacteria and 2 to 4-fold less than that of ofloxacin (**6**) against *staphylococci* and *streptococci* [214–216].

Its chemical name is 1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid. Its formula is shown in Fig. 9. In healthy volunteers, dose-dependent pharmacokinetics was seen after oral administration of 100, 200, 300, 400 or 500 mg of lomefloxacin (**26**) Morrison et al. [217]. The concentrations of lomefloxacin (**26**) in plasma and urine were determined by a sensitive and specific HPLC assay.

Briefly, following extraction into chloroform–iso-



(**26**)

Fig. 9. Chemical structure of lomefloxacin.

amyl alcohol (19:1, v/v) at pH 7.0 and evaporation, the residue was dissolved in a mobile phase (acetonitrile–0.05 *M* citric acid, 1.0 *M* ammonium acetate) and analyzed on a reversed-phase Nucleosil C_{18} column (250 mm×4.6 mm I.D.) with fluorescence detection (excitation, 280 nm; emission, 455 nm).

The assay precision ranged between 2.91 and 11.7% at the concentration tested. The plasma assay sensitivity was 0.010 $\mu g\,ml^{-1}$, and the urine assay sensitivity was 0.100 $\mu g\,ml^{-1}$.

Comparison of HPLC and microbiological assay for the determination of lomefloxacin (**26**) in biological fluids was developed by Shibl et al. [219].

Lomefloxacin (**26**) concentrations in plasma and in urine samples were determined by HPLC. Briefly, samples were analyzed by using a μBondapak C_{18} Radial–Pak Cartridge with Z-Module column after extraction at pH 7.0 with chloroform. The mobile phase consisted of a mixture prepared by mixing 230 ml, acetonitrile with 800 ml buffer (prepared by dissolving 2 g sodium acetate trihydrate, 2 g citric acid monohydrate, and 1 ml triethylamine in 1 liter distilled water). The mixture was adjusted to pH 4.8. The fluoroquinolone derivative of lomefloxacin (**26**) was used as an internal standard. The eluate was monitored fluorimetrically (280 nm excitation and 430 nm emission). The limit of detection was 0.1 $\mu g\,ml^{-1}$ for both plasma and urine.

In the microbiological assay, *E. coli* ATCC 1346 was the test organism using an agar diffusion technique. Good agreement was seen between the results of the two methods. Other HPLC methods have been reported for the analysis of lomefloxacin (**26**) in biological fluids e.g. serum, plasma, urine, erythrocyte [220–225].

Some of these methods have utilized UV detection [220–223], while others have utilized fluorescence detection [217,218,224,225]. Two of this methods have not included internal standards [218,224]. A method has been developed by Carlucci et al. [225] for the simultaneous determination of lomefloxacin (**26**), febufen and felbinac in human plasma.

A stereospecific HPLC assay of lomefloxacin (**26**) in human plasma was developed by Foster et al. [225]. Following addition of racemic acebutolol (internal standard), plasma samples were extracted at pH 7.0 with a mixture of chloroform–isopentyl alcohol–diethyl ether.

The organic layer was evaporated, and lomefloxacin and internal standard enantiomers in the resulting residue were derivatized with chloroform solutions of 1% triethylamine and 1% (*S*)-(+)-(1-naphthyl)ethyl isocyanate, followed by 2% ethylchloroformate one min later. Ethanolamine was added 30 s after the addition of ethylchloroformate.

The enantiomers were separate as diastereomers on an Radial Pak (100 mm×8) normal-phase column using a mobile phase of hexane–chloroform–methanol. The I.S. was detected by fluorescence at 245 and 420 nm (excitation and emission, respectively) during the first 12 min, after which time the wavelengths were 280 and 470 nm for detection of lomefloxacin (**26**). The method was sensitive and showed excellent linearity (10–1000 $ng\,ml^{-1}$) between added enantiomer concentrations and the peak-area ratio lomefloxacin-internal standard.

10. Rufloxacin

Rufloxacin (**27**) (MF 934) is a pyridobenzothiazine derivative with a fluorine atom and a methylpiperazine ring synthesized by Cecchetti et al. [226].

The chemical name of rufloxacin (**27**) is 9-fluoro-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de]-[1,4]-benzothiazine-6-carboxylic acid (Fig. 10).

This drug has been shown to possess marked in vivo activity against Gram-negative and Gram-posi-

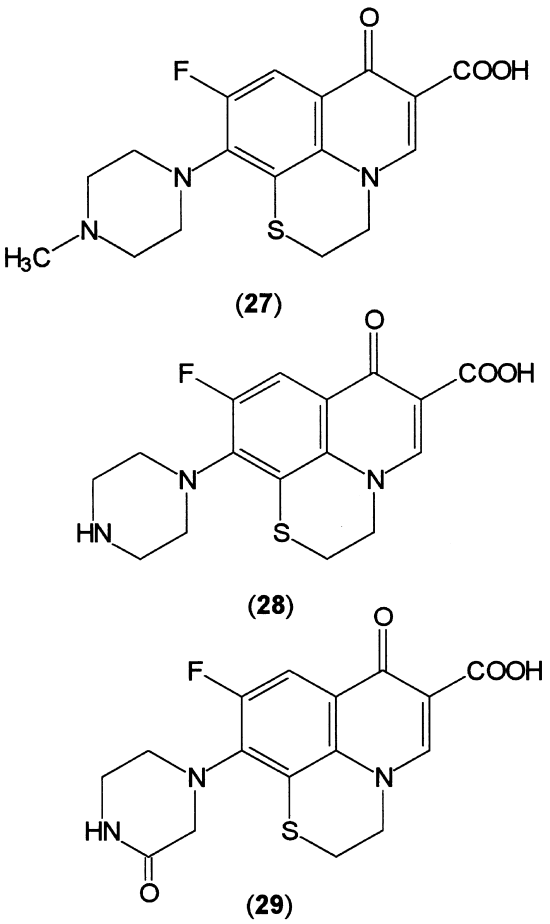


Fig. 10. Structures of rufloxacin and its metabolites.

tive bacteria [227,228]. Pharmacokinetic studies with both healthy subjects [229–232] and patients with lower respiratory tract infections [233,234] showed that rufloxacin (**27**) is eliminated slowly, with a plasma half-life of about 35 h.

The drug penetrates well into most tissue [232,235], and because of its long half-life, it can be administered once daily for the treatment of urinary [236] and respiratory [237] tract infections.

Many HPLC methods for the determination rufloxacin (**27**) in biological fluids have been reported [238–246]. A simple, specific, and sensitive HPLC method has been developed for routine monitoring in human serum and urine by Carlucci et al. [238]. Briefly, serum or urine spiked with internal standard pipemidic acid, were mixed with dichloromethane at pH 7.4. The evaporated extract was dissolved in mobile phase. The mobile phase consisted of a mixture of methanol–tetrabutylammonium iodide and 0.04 *M* phosphoric acid. Drugs were resolved on a 10 μm Viosfer LC–RP-18 column (250 mm×4.6 mm, I.D.) equipped with a guard column. The eluate was monitored at 295 nm. The detection limit of rufloxacin (**27**) was 0.05 $\mu g\,ml^{-1}$ for human serum and 0.03 $\mu g\,ml^{-1}$ for urine.

Other HPLC assays: Lombardi et al. [241], Vree et al. [242], Segre et al. [243], Carlucci et al. [240]. The separation and isolation of rufloxacin (**27**) from plasma and urine utilizing reversed-phase HPLC was accomplished by Segre et al. [243] The analysis was carried out using an isocratic elution technique.

Plasma samples after addition of deacetyldiazepam solution as an internal standard were extracted with chloroform. The organic phase was evaporated to dryness and then reconstituted with methanol. Separation was performed on a Nucleosil C_{18} column. The quantitation is achieved by monitoring the UV absorbance of the eluate at 245 nm. The same procedure was followed for urine samples.

An HPLC analysis was developed for the measurement of rufloxacin (**27**) and two of its possible metabolites *N*-demethylrufloxacin (**28**) and *N*-demethyloxorufloxacin (**29**) in plasma and urine samples by Vree et al. [242]. Plasma samples were deproteinized with 0.33 *M* perchloric acid and centrifuged, and aliquots of 20 μl of the supernatant was injected onto the column. The column was a CpSpher 5ODS 5 μm (250 mm×4.6 mm, I. D.) with a guard column (10 μm particle size, 75 mm×2.1 mm, I. D.) packed with pellicular reversed-phase. The analysis was carried out using a ternary HPLC pump in a gradient elution technique.

Urine samples were diluted 10 times with distilled water and 20 μl was injected onto the column. A method has been developed by Lombardi et al. [241] for the detection and analysis of rufloxacin (**27**) and its main active metabolite in plasma, urine and bile. A 500 μl volume of plasma sample was pipetted into a 1.5 ml microtest-tube and 25 μl of the internal standard (pipemidic acid) and 50 μl of 70% perchloric acid were added. After vortex-mixing, sonicating and centrifuging, the supernatant was transferred to an autosampler vial. Urine samples were diluted with water. This was transferred to an

autosampler vial and 10 μl were injected for analysis while for bile a 500 μl volume of each sample was pipetted into a 1.5 ml microtest-tube and the tubes were centrifuged. The supernatant was filtered through a 0.2 μm filter, transferred to an autosampler vial and aliquot of 5 μl was injected.

For the determination of rufloxacin (**27**) and *N*-demethyl metabolite (**28**) in plasma and urine when is required, a structurally related internal standard was used. For bile and urine, the use of an internal standard was not required as rufloxacin (**27**) and *N*-demethyl metabolite (**28**) are completely recovered. The limit of detection was 10 ng ml^{-1} for both rufloxacin (**27**) and *N*-desmethyl metabolite (**28**) in plasma, urine and bile.

The separation and isolation of rufloxacin (**27**) in serum and urine utilizing anion-exchange HPLC was accomplished by Carlucci et al. [240]. The analysis was carried out using a mixture of acetonitrile–0.05 *M* phosphate buffer (pH 7.0). The column eluate was monitored at 296 nm. The ofloxacin (**6**) was used as an internal standard. The detection limits for serum and urine were 0.1 and 0.05 μg ml^{-1}, respectively.

A number of authors have shown that certain quinolones have a pharmacokinetic interaction with theophylline, owing to a metabolic inhibition with variation of the total clearance of theophylline from 43 to 75%, depending upon the dose.

A HPLC method for the simultaneous quantification of theophylline and rufloxacin (**27**) in plasma, which utilizes a small sample volume in order to use it routinely in clinical practice for therapeutic drug monitoring has been developed by Carlucci et al. [244]. Liquid–liquid extraction was used for the sample preparation. Fenbufen is one of the non steroidal antiinflammatory, antipyretic and analgesic agents that belongs to the group of propionic acid derivative; it has been frequently used because of its relative low gastric toxicity. This drug is readily transformed in its metabolite bifenylacetate, felbinac.

All the quinolones inhibit the γ-aminobutyric acid (GABA) receptors binding to the synaptic membranes in a dose dependent manner [246]. It was reported that some quinolones induced severe convulsions. Fenbufen, but more so its metabolite felbinac, showed a marked intensification of this neurotoxic effect. A quantitative anion-exchange HPLC method for the determination of rufloxacin (**27**) and fenbufen in plasma is described by Carlucci et al. [247]. Briefly, samples were analyzed by using a Zorbax SAX column after extraction with a mixture of dichloromethane–diethyl ether. The mobile phase consisted of a mixture of acetonitrile-and 0.1 *M* phosphate buffer (pH 7.0). An antiinflammatory analog of fenbufen was used as an internal standard. The limit of detection was 0.1 μg ml^{-1} for rufloxacin (**27**) 0.3 μg ml^{-1} for fenbufen and 0.1 μg ml^{-1} for felbinac, respectively.

11. Conclusion

The fluoroquinolones are potent synthetic agents active against a variety of bacterial species. HPLC has proven to be widely applicable to the determination of fluoroquinolones in body fluids. Workable HPLC methods have been reported for all but a few clinically important fluoroquinolones. HPLC determinations are sensitive, selective, reproducible, accurate and convenient. The particular advantage of HPLC relative to the other widely available techniques for fluoroquinolones determinations is its chemical specificity. Isocratic reversed-phase HPLC seemed the preferable approach for several fluoroquinolones.

Addition of tetrabutylammonium phosphate or another amine appeared essential in order to avoid tailing. Stationary phases with a high degree of end-capping, such as Nucleosil C_{18} 5 μm were preferred. Methylpiperazine quinolones were difficult to separate from their demethyl metabolites by reversed-phase HPLC. But ion chromatography reversed-phase combined with a RP precolumn produced a different elution pattern with complete separation of ofloxacin (**6**) and fleroxacin (**20**) from their *N*-oxide and demethyl metabolite. With fluorescence detectors, separation from endogenous compounds was generally good.

Several other drugs produce fluorescent peaks, and need checking for possible interference under the individual chromatographic conditions used. Most methods allow the simultaneous detection of some metabolites similar in polarity to the parent drug.

For other metabolites, *N*-sulfociprofloxacin (**10**), and oxociprofloxacin (**11**), separate chromatographic runs with modified mobile phase were warranted.

The separation of ofloxacin enantiomers is achievable by derivatization or by use a column containing bovine serum albumin bound to silica. The intrinsic fluorescence of the quinolones makes them good candidates for fluorescence detection.

Fluorescence detection was highly sensitive and specific, and also gave good precision and a wide range of linear detector response, it is obligatory if only small sample volumes are available and for clinical specimens where interferences are likely to be present. Although in most studies apparent recoveries from 85 to 105% were reported, the accuracy of fluorimetric determination of quinolones in serum remains a critical issue. In a comparative study the reported recoveries from serum were 101–110% for two HPLC methods and 109–120% for two microbiological methods [98]. Fluorescence yield is strongly influenced by the matrix, which if slightly different between standards and samples my cause systematic errors. The fluorescence yield of metabolites with a modified piperazine ring is altered, usually downwards. Thus fluorescence detection does not allow the determination of all metabolites, although it is achievable for all known ciprofloxacin metabolites by photothermal derivatization [145].

The UV absorbance detector serves mainly for non fluorescent metabolites, although its sensitivity is much lower [156], it is also less specific since many endogenous compounds absorb in the 254–270 nm range. It has been advocated [132] for the parent compound also, but the sample size was rather larger and the apparent recovery from serum was only 60%. While absorbance detection appears poorly suitable for therapeutic monitoring, the diode array detectors seems adapt for identifying metabolites. Comparison was mainly with microbiological assay. Accord between HPLC and bioassay depends on the absence of active metabolites, thus demethylofloxacin (**7**) is still microbiologically active. However, in healthy volunteers the two methods gave identical results for ofloxacin (**6**) in serum and urine, which contain very low concentrations of metabolites.

For ciprofloxacin (**9**), which might give active metabolites in urine, bioassay results were higher. In the case of serum, wherein metabolite concentrations are very low, other factors evidently operate, the discrepancy has been observed also by several authors [91,98]. In conclusion, HPLC has been proven in many laboratories to be an effective tool for therapeutic monitoring of fluoroquinolones. Due to the inherent power of HPLC and the impact of better methods and equipment as detailed above, the role of HPLC in the therapeutic monitoring of fluoroquinolones may be expected to increase during the coming years.

References

[1] R. Albrecht, Progr. Drug Res. 21 (1977) 9.
[2] D.C. Hooper, J.X. Wolfson, Antimicrob. Agents Chemother. 28 (1985) 716.
[3] J.X. Wolfson, D.C. Hooper, Antimicrob. Agents Chemother. 28 (1985) 581.
[4] N. Hoiby, Eur. J. Clin. Microb. 5 (1986) 138.
[5] R. Janknegt, Pharm. Week Sci. Ed. 8 (1986) 1.
[6] H.C. Neu, Pharmacol. Ther. 41 (1989) 207.
[7] P.B. Fernandes, J. Clin. Pharmacol. 28 (1988) 156.
[8] H.J. Paton, D.S. Reeves, Drugs 36 (1988) 193.
[9] J. Schentag, J. Domagala, Res. Clin. Forums 7 (1985) 9.
[10] J.B. Cornett, M.P. Wentland, Ann. Rept. Med. Chem. 21 (1986) 139.
[11] D.T.W. Chu, P.B. Fernandes, Antimicrobial. Agents Chemother. 33 (1989) 131.
[12] L.A. Mitscher, P.V. Devasthale, R.M. Zavod, in: G.C. Crumplin (Ed.), The 4-Quinolones: Antibacterial Agents In Vitro, Springer-Verlag, Berlin, 1990, p. 115.
[13] V.T. Andriole, The quinolones, Academic Press, London, 1988.
[14] J.X. Wolfson, D.C. Hooper, Clin. Microbiol. Rev. 2 (1989) 378.
[15] L.A. Mitscher, P.N. Sharm, D.T.W. Chu, L.L. Shen, A.G. Pernet, J. Med. Chem. 29 (1986) 2044.
[16] L.A. Mitscher, P.N. Sharm, L.L. Shen, D.T.W. Chu, J. Med. Chem. 30 (1987) 2283.
[17] M.M. Zweerink, A. Edison, Antimicrob. Agents Chemother. 29 (1986) 598.
[18] L.L. Shen, L.A. Mitsher, P.N. Sharma, T.J. O'Donnel, D.T.W. Chu, C.S. Cooper, T. Rosen, A.P. Pernet, Biochem. 28 (1989) 3886.
[19] W. Sadée, G.C.M. Beelen, Drug Level Monitoring, Wiley, Chichester, 1980.
[20] I.N. Papadoyannis, HPLC in Clinical Chemistry, Marcel Dekker, New York, 1990.
[21] E.T. Lin, W. Sadée, Drug Level Monitoring, Wiley, Chichester, 1986.
[22] G. Carlucci, Cromatografia Liquida ad Alta Efficienza Applicazione all'Analisi di Farmaci in Matrici Biologiche, Benedetti, L'Aquila, 1997.
[23] J. Chamberlain, Analysis of Drugs in Biological Fluids, CRC Press, Boca Raton, 1985.

[24] D.J. Griggs, R. Wise, J. Antimicrob. Chemother. 24 (1989) 437.
[25] C.Y. Chan, A.W. Lam, G.L. French, J. Antimicrob. Chemother. 23 (1989) 597.
[26] H. Lode, G. Hoffken, M. Boeckk, N. Deppermann, K. Borner, P. Koeppe, J. Antimicrob. Chemother. 26 (1990) 415.
[27] A. Nangia, F. Lam, C.T. Hung, J. Pharm. Sci. 79 (1990) 988.
[28] H. Koga, A. Itoh, S. Murayama, S. Suzue, T. Irikura, J. Med. Chem. 23 (1980) 1358.
[29] M. Eandi, I. Viano, F. Di Nola, L. Leone, E. Genazzani, Eur. J. Clin. Microbiol. 2 (1983) 253.
[30] L.T. Pauliukonis, D.G. Musson, W.F. Bayne, J. Pharm. Sci. 73 (1984) 99.
[31] S.J. Morton, V.H. Shull, J.D. Dick, Antimicrob. Agents Chemother. 30 (1986) 325.
[32] F. Gutzler, J.X. de Vries, Fortschr. Antimikr. Antineoplast. Chemother. 3 (1984) 673.
[33] R. Warlich, D. Krauß, E. Mutschler, Arzneim.-Forsch. Drug Res. 39 (1989) 656.
[34] W. Schoenfeld, J. Knoeller, K.D. Bremm, A. Dahlhoff, B. Weber, W. Koenig, Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A. 261 (1986) 338.
[35] D. Xu, Y. Yuan, X. Tu, Yaowu Fenxi Zazhi 10 (1990) 265.
[36] T. Ozaki, H. Uchida, T. Irikura, Chemotheraphy 29 (1981) 128.
[37] A.J.N. Groeneveld, J.R.B.J. Brouwers, Pharm. Weekbl. Sci. 8 (1986) 79.
[38] I. Nilsson-Ehle, J. Chromatogr. 416 (1987) 207.
[39] A. Tilly Melin, M. Ljungerantz, G. Schill, J. Chromatogr. 185 (1979) 225.
[40] A. Laganà, R. Curini, G. D'Ascenzo, A. Marino, M. Rotatori, J. Chromatogr. 417 (1987) 135.
[41] V.K. Boppana, B.N. Swanson, Antimicrob. Agents Chemother. 21 (1982) 808.
[42] B. Joos, B. Lederberger, M. Flepp, J.D. Bettex, R. Lüthy, W. Siegenthaler, Antimicrob. Agents Chemother. 27 (1985) 353.
[43] M.S. Hussain, V. Chukwumaeze-Obiajunwa, R.G. Micetich, J. Chromatogr. B 663 (1995) 379.
[44] A. Laganà, A. Marino, M. Rotatori, R. Curini, G. D'Ascenzo, L. Miano, J. Pharm. Biomed. Anal. 6 (1988) 221.
[45] C. Forchetti, G. Carlucci, G. Cavicchio, M. Bologna, J. Chromatogr. 309 (1984) 177.
[46] S.C. Wallis, B.G. Charles, L.R. Gahan, J. Chromatogr. B 674 (1995) 306.
[47] D.J. Edwards, S.K. Bowles, C.K. Svensson, M.J. Rybak, Clin. Pharmacokin. 15 (1988) 194.
[48] W.J.A. Wijnands, T.B. Vree, C.L.A. van Herwaarden, Br. J. Clin. Pharmacol. 22 (1986) 677.
[49] J.D. Davis, L. Aarons, J.B. Houston, J. Chromatogr. 621(9) (1993) 105.
[50] G. Carlucci, P. Mazzeo, G. Palumbo, Biomed. Chromatogr. 7 (1993) 126.
[51] Y. Katagiri, K. Naora, N. Ichikawa, M. Hayashibara, K. Iwamoto, Chem. Pharm. Bull. 37 (1989) 2858.
[52] A.I. Hayakawa, T. Hiramitsu, Y. Tanaka, Chem. Phar. Bull. 32 (1984) 4907.
[53] B.D. Leigh, R. Finch, R. Spencer, J. Antimicrob. Chemother. 22 (1988) 1.
[54] R.C. Moellering, H. Neu, Am. J. Med. 87 (1989) 1.
[55] L.O. White, A.P. MacGowan, A.M. Lovering, D.S. Reeves, I.G. Mackay, Drugs 34 (1987) 56.
[56] K. Sudo, K. Hashimoto, T. Kurata, O. Okazaki, M. Tsumura, H. Tachizawa, Chemotheraphy (Tokyo) 32 (1984) 1203.
[57] J.B.J. Borema, Pharm. Weekb Sci. 8 (1986) 46.
[58] B.I. Davis, F.PV. Maesen, J.P. Teengs, C. Baur, Phar. Weekb Sci. 8 (1986) 53.
[59] H. Lode, G. Höffken, P. Olschewski, B. Sievers, A. Kirch, K. Borner, P. Koeppe, Antimicrob. Agents Chemother. 31 (1987) 1338.
[60] G. Carlucci, S. Guadagni, G. Palumbo, J. Liq. Chromatogr. 9 (1986) 2539.
[61] Y. Katagiri, K. Naora, N. Ichikawa, M. Hayashibara, K. Iwamoto, J. Chromatogr. 431 (1988) 135.
[62] K. Matsubayashi, T. Une, Y. Osada, J. Chromatogr. 495 (1989) 354.
[63] D.J. Griss, R.A. Wise, J. Antimicrob. Chemother. 24 (1989) 437.
[64] T. Ohkubo, M. Kudo, K. Sugawara, Anal. Sci. 7 (1991) 741.
[65] T. Ohkubo, M. Kudo, K. Sugawara, J. Chromatogr. 573 (1992) 289.
[66] D.R.P. Guay, J.A. Apsahl, F.G. McMahon, R. Vargas, G.R. Matzke, S. Flor, Antimicrob. Agents Chemother. 36 (1992) 308.
[67] K. Borner, H. Hartwig, H. Lode, H. Hoeffken, Fresenius' Z Anal. Chem. 324 (1986) 355.
[68] E. Kraas, A. Hirrle, Fresenius' Z Anal. Chem. 324 (1986) 354.
[69] L.J. Notarianni, R.W. Jones, J. Chromatogr. 431 (1988) 461.
[70] A. Le Coguic, R. Bidault, R. Farinotti, A. Dauphin, J. Chromatogr. 434 (1988) 320.
[71] A. Mignot, M.A. Lefebvre, J.B. Fourtillan, J. Chromatogr. 430 (1988) 192.
[72] J. Knöller, W. König, W. Schönfeld, K.D. Bremm, M. Köller, J. Chromatogr. 427 (1988) 257.
[73] K.H. Lehr, P. Damm, J. Chromatogr. 425 (1988) 153.
[74] N. Bitar, R. Clases, P. Van der Auwera, Antimicrob. Agents Chemother. 33 (1989) 1686.
[75] R. Warlich, H.C. Korting, M. Schäfer, E. Mutschler, Antimicrob. Agents Chemother. 34 (1990) 78.
[76] N. Miyazawa, T. Uematsu, A. Mizuno, S. Nagashima, M. Nagashima, Forensic Sci. Int. 51 (1991) 65.
[77] O. Okazaki, H. Aoki, H. Hakusui, J. Chromatogr. 563 (1991) 313.
[78] T. Uematsu, Forensic Sci. Int. 63 (1993) 261.
[79] A. Mizuno, T. Uematsu, N. Nakashima, J. Chromatogr. B 653 (1994) 187.
[80] F. Koizumi, A. Ohnishi, H. Takemura, S. Okubo, T. Kagami, T. Tanaka, Antimicrob. Agents Chemother. 38 (1994) 1140.
[81] S. Zeng, L. Zhang, Z.Q. Liu, Yao Hseeh Hsueh Pao 29 (1994) 233.
[82] D. Fabre, F. Bressolle, J.M. Kinowski, O. Bouvet, F. Paganin, M. Galtier, J. Pharm. Biomed. Anal. 12 (1994) 1463.

[83] T. Ohkubo, M. Kudo, K. Sugawara, Y. Sawada, J. Pharm. Pharmacol. 46 (1994) 522.
[84] J. Macek, P. Ptácek, J. Chromatogr. B 673 (1995) 316.
[85] R.J. Perkins, W. Liu, G. Drusano, A. Madu, M. Mayers, C. Madu, M.H. Miller, Antimicrob Agents Chemother. 39 (1995) 1493.
[86] M. Fresta, A. Spadaro, G. Cerniglia, I.M. Ropero, G. Puglisi, P.M. Furneri, Antimicrob. Agents Chemother. 39 (1995) 1372.
[87] T. Uematsu, K. Kosuge, S.I. Araki, M. Ishiye, Y. Asai, M. Nakashima, Ther. Drug. Monit. 17 (1995) 101.
[88] C.V.S. Subrahmanyam, S.E. Reddy, M.S. Redd, Indian Drug 33 (1996) 76.
[89] A.L. Barry, R.N. Jones, J. Antimicrob. Chemother. 23 (1989) 527.
[90] T. Bergan, A. Dalhoff, R. Rohwedder, Infection 16 (1988) S3.
[91] B. Joos, B. Lederberger, M. Flepp, J.D. Bettex, R. Lüthy, W. Siegenthaler, Antimicrob. Agents Chemother. 27 (1985) 353.
[92] I.W. Fong, W.H. Ledbetter, A.C. Vandenbroucke, M. Simbul, V. Rahm, Antimicrob. Agents Chemother. 29 (1986) 405.
[93] A.L. Barry, R.N. Jones, C. Thornsberry, L.W. Ayers, E.H. Gerlach, H.M. Sommers, Antimicrob. Agents Chemother. 25 (1984) 633.
[94] A.L. Barry, R.N. Jones, Antimicrob. Agents Chemother. 25 (1984) 775.
[95] A.L. Barry, R.J. Fass, J.P. Anhalt, H.C. Neu, C. Thornsberry, R.C. Tilton, B.G. Painter, J.A. Washington II, J. Clin. Microbiol. 21 (1985) 880.
[96] H. Giamarellou, E. Daphnis, C. Dendrinos, G.K. Daikos, Drugs Exptl. Clin. Res. 11 (1985) 351.
[97] R. Wise, R.M. Lockley, M. Webberly, J. Dent, Antimicrob. Agents Chemother. 26 (1984) 208.
[98] A. Weber, A.L. Smith, K. Wong, B. Painter, G. Krol, Meths. Findings Exptl. Clin. Pharmakol. 10 (1988) 123.
[99] M.A. Gonzales, A.H. Moranchel, S. Duran, A. Pichardo, J.L. Magana, B. Painter, A. Forrest, G.L. Drusano, Antimicrob. Agents Chemother. 28 (1985) 235.
[100] M.A. Gonzales, F. Uribe, S.D. Moisen, A.P. Fuster, A. Selen, P.G. Welling, B. Painter, Antimicrob. Agents Chemother. 26 (1984) 741.
[101] T.A. Tartaglione, A.C. Raffalovich, W.J. Poynor, A. Espinel-Ingroff, T.M. Kerkering, Antimicrob. Agents Chemother. 29 (1986) 62.
[102] T. Bergan, C. Delin, S. Johansen, I.M. Kolsrad, C.E. Nord, S.B. Thorstiensson, Antimicrob. Agents Chemother. 29 (1986) 298.
[103] M.J. Smith, L.O. White, H. Bowyer, M.E. Hodson, J.C. Batten, Antimicrob. Agents Chemother. 30 (1986) 614.
[104] E. Goormans, A. Dalhoff, B. Kazzaz, J. Branolte, Chemotherapy 32 (1986) 7.
[105] J.T. Lettieri, M.C. Rogge, L. Kaiser, R.M. Echols, A.H. Heller, Antimicrob. Agents Chemother. 36 (1992) 993.
[106] A. Dalhoff, H.U. Eickenberg, Infection. 13 (1985) 78.
[107] H.M. Siefert, D. Maruhm, W. Maul, D. Forster, W. Ritter, Arzneim-Forsh. Drug Res. 36 (1986) 1496.
[108] G.L. Drusano, Am. J. Med. 82 (1987) 339.
[109] I. Shalit, R.B. Greenwood, M.I. Marks, J.A. Pederson, D.L. Frederick, Antimicrob. Agents Chemother. 30 (1986) 152.
[110] M. Dan, T. Zuabi, C. Quassem, H.H. Rotmensch, Antimicrob. Agents Chemother. 36 (1992) 677.
[111] D.M. Campoli-Richards, J.P. Monk, A. Price, P. Benfield, P.A. Todd, A. Ward, Drugs 35 (1988) 373.
[112] K.I. Plaisance, G.L. Drusano, A. Forrest, C.I. Bustamante, H.C. Standiford, Antimicrob. Agents Chemother. 31 (1987) 956.
[113] H. Peltola, M. Vaarala, O.V. Renkonen, P.J. Neuvonen, Antimicrob. Agents Chemother. 36 (1992) 1086.
[114] R.E. Brjant, A.I. Hartstein, Int. J. Clin. Pharm. Res. 3 (1987) 187.
[115] A. Dalhoff, W. Weidner, Eur. J. Clin. Microbiol. 3 (1984) 360.
[116] B. Ledergerber, J.D. Bettex, B. Joos, M. Flepp, R. Luthy, Antimicrob. Agents Chemother. 27 (1985) 350.
[117] T.C. Gasser, S.C. Ebert, P.H. Graversen, P.O. Madsen, Antimicrob. Agents Chemother. 31 (1987) 709.
[118] K.E. Aldridge, D.D. Schiro, L. Tsai, A. Janney, C.V. Sanders, R.L. Marier, Cur. Ther. Res. 37 (1985) 754.
[119] B. Chattopadhyay, J.C. Teli, J. Antimicrob. Chemother. 14 (1984) 191.
[120] S. Esposito, D. Galante, D. Barba, G. D'Errico, A. Mazzone, S. Montanaro, Int. J. Clin. Pharm. Res. 7 (1987) 181.
[121] J.B.J. Boerema, A. Dalhoff, F.M.J. Debruyne, Chemotherapy 31 (1985) 13.
[122] J.D. Klinger, S.C. Aronoff, J. Antimicrob. Chemother. 15 (1985) 679.
[123] W. Wingender, K.H. Graefe, W. Gau, D. Förster, D. Beermann, P. Schacht, Eur. J. Clin. Microbiol. 3 (1984) 355.
[124] M. Dan, J. Golomb, A. Gorea, Z. Braf, S.A. Berger, Antimicrob. Agents Chemother. 30 (1986) 88.
[125] M. LeBel, M.G. Bergeron, F. Vallé, C. Fiset, G. Chassé, P. Bigonnesse, G. Rivard, Antimicrob. Agents Chemother. 30 (1986) 260.
[126] J.M. Brogard, F. Jehl, H. Monteil, M. Adloff, J.F. Blickle, P. Levy, Antimicrob. Agents Chemother. 28 (1985) 311.
[127] R.L. Davis, J.R. Koup, J. Williams-Warren, A. Weber, L. Heggen, D. Stempel, A.L. Smith, Antimicrob. Agents Chemother. 31 (1987) 915.
[128] H. Tanimura, S. Tominaga, F. Rai, H. Matsumoto, Arzneim. Forsch. Drug Res. 36 (1986) 1417.
[129] E. Vallée, E. Azoulay-Dupuis, J. Bauchet, J.J. Pocidalo, J. Pharm. Exp. Ther. 262 (1992) 1203.
[130] A.R. Abadia, L. De Francesco, A. Guaitani, Drug Metab. Dispos. 23 (1995) 197.
[131] F. Jehl, C. Gallion, J. Debs, J.M. Brogard, H. Monteil, R. Mink, J. Chromatogr. 339 (1985) 347.
[132] F. Vallée, M. Lebel, M.G. Bergeron, Ther. Drug Monitor. 8 (1986) 340.
[133] K. Tyczkowska, K.M. Hedeen, D.P. Aucoin, A.L. Aronson, J. Chromatogr. 493 (1989) 337.
[134] G. Mack, J. Chromatogr. 582 (1992) 263.
[135] A.C. Mehta, S. Hart-Davies, E.A. Kay, J. Clin. Phar. Ther. 17 (1992) 117.

[136] L. Pou-Clave, F. Campos-Barreda, C. Pascual-Mostaza, J. Chromatogr. 563 (1991) 211.
[137] D. Hoffler, A. Dalhoff, W. Gau, D. Beerman, A. Michl, Eur. J. Clin. Microbiol. 3 (1984) 363.
[138] A. Shah, J. Lettieri, D. Nix, J. Wilton, H. Heller, Antimicrob. Agents Chemother. 39 (1995) 1003.
[139] I. Nilsson-Ehle, J. Chromatogr. 416 (1987) 207.
[140] K. Borner, H. Lode, G. Hoffken, C. Prinzing, P. Glatzel, R. Wiley, J. Clin. Chem. Clin. Biochem. 24 (1986) 325.
[141] C.M. Myers, J.L. Blumer, J. Chromatogr. 422 (1987) 153.
[142] Y. Katagiri, K. Naora, N. Ichikawa, M. Hayashibara, K. Iwamoto, Chem. Pharm. Bull. 38 (1990) 2884.
[143] Z. Li, P. Guo, Q. Guo, Yaowu Fenxi Zazhi 14 (1994) 16.
[144] G.J. Krol, G.W. Beck, T. Benham, J. Pharm. Biomed. Anal. 14 (1995) 181.
[145] H. Scholl, K. Schimidt, B. Weber, J. Chromatogr. 416 (1987) 321.
[146] W.M. Awni, J. Clarkson, D.R.P. Guay, J. Chromatogr. 419 (1987) 414.
[147] S.J. Morton, V.H. Shull, J. Dick, Antimicrob. Agents Chemoter. 30 (1986) 325.
[148] A. El-Yazigi, S. Al-Rawithy, Ther. Drug Monit. 12 (1990) 378.
[149] N.E. Basci, A. Bozkurt, D.D. Kalayci, S.O. Kayaalp, J. Pharm. Biomed. Anal. 14 (1996) 353.
[150] G.J. Krol, A.J. Noe, D. Beermann, J. Liq. Chromatogr. 9 (1986) 2897.
[151] M. Lovdahl, J. Steury, H. Russlie, D.M. Canafax, J. Chromatogr. 617 (1993) 329.
[152] W. Gau, H.J. Ploschke, K. Schmidt, B. Weber, J. Liq. Chromatogr. 8 (1985) 485.
[153] D.E. Nix, J.M. De Vito, J.J. Schentag, Clin. Chem. 31 (1985) 684.
[154] C.E. Fasching, L.R. Peterson, J. Liq. Chromatogr. 8 (1985) 555.
[155] L. Drusano, H.C. Standiford, K. Plaisance, A. Forrest, J. Leslie, J. Caldwell, Antimicrob. Agents Chemother. 30 (1986) 444.
[156] A. Weber, D. Chaffin, A. Smith, K.E. Opheim, Antimicrob. Agents Chemother. 27 (1985) 531.
[157] S.P. Zhai, M.R. Korrapati, X.X. Wei, S. Muppalla, S.P. Vestal, J. Chromatogr. 669 (1995) 372.
[158] C.H. Fenlon, M.H. Cynamon, Antimicrob. Agents Chemother. 29 (1986) 386.
[159] B. Kemmerich, G.J. Small, J.E. Pennington, Antimicrob. Agents Chemother. 29 (1986) 395.
[160] W. Beheins-Baumann, J. Martell, Chemotherapy 33 (1987) 328.
[161] G.L. Drusano, K.I. Plaisance, A. Forrest, H.C. Standiford, Antimicrob. Agents Chemother. 30 (1986) 440.
[162] K. Naora, Y. Katagiri, N. Ichikawa, M. Hayashibara, K. Iwamoto, J. Chromatogr. 530 (1990) 186.
[163] N.E. Bozkurt, D. Kalayci, S.O. Kayaalp, J. Pharm. Biomed. Anal. 14 (1996) 353.
[164] H. Stuht, H. Lode, P. Koeppe, K.L. Rost, T. Schagerg, Antimicrob. Agents Chemother. 39 (1995) 1045.
[165] L.K. Jim, N. El-Sayed, K.I. Al-Khamis, J. Clin. Pharm. Ther. 17 (1992) 111.
[166] M.P. Weinstein, R.G. Deeter, K.A. Swanson, J.S. Gross, Antimicrob. Agents Chemother. 35 (1991) 2352.
[167] G.L. Drusano, M. Weir, A. Forrest, K. Plaisance, T. Emm, H.C. Standiford, Antimicrob. Agents Chemother. 31 (1987) 860.
[168] F. Kees, W. Raasch, H. Grobecker, Arzneim.-Forsch. Drug Res. 42 (1992) 570.
[169] M.N. Dundley, J. Ericson, S.H. Zinner, Antimicrob. Agents Chemother. 31 (1987) 1782.
[170] W. Gau, J. Kurz, U. Petersen, H.J. Ploschke, C. Wuensche, Arzneim.-Forsch. Drug Res. 36 (1986) 1545.
[171] G. Montay, Y. Goueffon, F. Roquet, Antimicrob. Agents Chemother. 25 (1984) 463.
[172] B. Lacarelle, C. Le Guellec, A. Morel, J. Albanese, M. Alazia, M. Ballarau, M. Llurens, R. Bruno, G. Francois, A. Durand, Ther. Drug Monit. 16 (1994) 209.
[173] N. Janin, H. Meugnier, J.F. Desnottes, R. Woehrle, J. Fleurette, Antimicrob. Agents Chemother. 31 (1987) 1665.
[174] G. Montay, B. Blain, F. Roquet, A. Le Hir, J. Chromatogr. 273 (1983) 359.
[175] G. Montay, J.P. Tassel, J. Chromatogr. 339 (1985) 214.
[176] C.Y. Chan, A.W. Lam, G.L. French, J. Antimicrob. Chemother. 23 (1989) 597.
[177] D.J. Griggs, R. Wise, J. Antimicrob. Chemother. 24 (1986) 437.
[178] R.Q. Xu, Z.L. Xia, S. Guo, SEPU 13 (1995) 47.
[179] N. Abanmi, I. Zaghloul, N. El Sayed, K. Al Khamis, Ther. Drug Monit. 18 (1996) 158.
[180] Ministry of health and Welfare. Information on Drug Adverse Reactions, Tokyo, 81 (1986) 1.
[181] G. Carlucci, G. Palumbo, P. Mazzeo, J. Liq. Chromatogr. Rel. Technol. 19 (1996) 1107.
[182] M.P. Wentland, D.M. Bailey, J.B. Cornett, R.A. Dobson, R.G. Powles, R.B. Wagner, J. Med. Chem. 27 (1984) 1103.
[183] H.C. Neu, P. Labthavikul, Diagn. Microbiol. Infect. Dis. 3 (1985) 469.
[184] J.A. Johnson, D.P. Benziger, Antimicrob. Agents Chemother. 27 (1985) 774.
[185] D.J. Pohlod, L.D. Saravolatz, Antimicrob. Agents Chemother. 25 (1984) 377.
[186] L.F. MeCoy, P.B. Crawmer, D.P. Benziger, Antimicrob. Agents Chemother. 27 (1985) 769.
[187] B.P. Crawmer, J.A. Cook, R.R. Brown, J. Chromatogr. 530 (1990) 407.
[188] N. Chin, D.C. Brittain, H.C. Neu, Antimicrob. Agents Chemother. 29 (1986) 675.
[189] K. Hirai, H. Aoyama, M. Hosaka, Y. Oomori, Y. Niwata, S. Suzue, T. Irikura, Antimicrob. Agents Chemother. 29 (1986) 1059.
[190] N. Manek, J.M. Andrews, R. Wise, Antimicrob. Agents Chemother. 30 (1986) 330.
[191] M. Nakashima, M. Kanamuru, T. Uematu, A. Takiguchi, A. Mizuno, T. Itaya, F. Kawahara, T. Ooie, S. Saito, H. Uchida, K. Masuzawa, J. Antimicrob. Chemother. 22 (1988) 133.
[192] E. Weidekamm, R. Portmann, K. Suter, C. Partos, D. Dell, P.W. Lücker, Antimicrob. Agents Chemother. 31 (1987) 1919.

[193] R. Wise, B. Kirkpatrick, J. Ashby, D.J. Griggs, Antimicrob. Agents Chemother. 31 (1987) 161.
[194] H. Kusajima, T. Ooie, F. Kawahara, H. Uchida, J. Chromatogr. 381 (1986) 137.
[195] W.M. Awni, J.A. Maloney, K.L. Heim-Duthoi, Clin. Chem. 34 (1988) 2330.
[196] D. Dell, C. Partos, R. Portmann, J. Liq. Chromatogr. 11 (1988) 1299.
[197] R. Wise, B. Kirkpatrick, J. Ashby, D.J. Griggs, Antimicrob. Agents Chemother. 31 (1987) 161.
[198] F. Sörgel, R. Seelmann, K. Naber, R. Metz, P. Muth, J. Antimicrob. Chemother. 22 (1988) 169.
[199] E. Brunt, J. Limberg, H. Derendorf, J. Pharm. Biomed. Anal. 8 (1990) 67.
[200] P. Heizmann, D. Dell, H. Eggers, R. Gora, J. Chromatogr. 527 (1990) 91.
[201] H. Kusajima, N. Ischikawa, M. Macnida, H. Hushida, T. Irikura, Antimicrob. Agents Chemother. 30 (1986) 304.
[202] D.T.W. Chu, P.B. Fernandes, A.K. Claiborne, E. Pihuleac, C.W. Nordeen, R.E. Maleczka, A.G. Pernet, J. Med. Chem. 28 (1985) 1558.
[203] A.L. Barry, in: P.B. Fernades (Ed.), International Telesymposium on Quinolones, J.R. Prous, Barcelona, 1989, p. 237.
[204] J.M. Stamm, C.W. Hanson, D.T.W. Chu, R. Bailer, C. Vojtko, P.B. Fernades, Antimicrob. Agents Chemother. 32 (1986) 193.
[205] P.B. Fernandes, N. Shipkowtiz, R.R. Bower, K.P. Jarvis, J. Weisz, D.T.W. Chu, J. Antimicrob. Chemother. 18 (1986) 693.
[206] P.B. Fernades (Ed.), International Telesymposium on Quinolones, J.R. Prous, Barcelona, 1989, p. 1
[207] D.T.W. Chu, G.R. Granneman, P.B. Fernades, Drugs Future 10 (1985) 543.
[208] P.B. Fernades, D.T.W. Chu, R.N. Swanson, N.R. Ramer, C.W. Hanson, R.R. Bower, J.M. Stamm, D.J. Hardy, Antimicrob. Agents Chemother. 32 (1988) 27.
[209] G.R. Granneman, K.M. Snyder, V.S. Shu, Antimicrob. Agents Chemother. 30 (1986) 689.
[210] G.R. Granneman, L.T. Sennello, J. Chromatogr. 413 (1987) 199.
[211] G. Ping, L. Zhang-wan, W. Tie-seng, Yaowu Fenxi Zazhi 12 (1992) 1.
[212] N.X. Chin, V.M. Figueredo, A. Novelli, H. Neu, Eur. J. Microbiol. Infect. Did. 259 (1988) 13560.
[213] T. Hirose, E. Okeazaki, H. Kato, Y. Ito, M. Inoue, S. Mitsuhashi, Antimicrob. Agents Chemother. 31 (1987) 854.
[214] R. Edwards, M. Kanematsu, D. Greenwood, J. Antimicrob. Chemother. 22 (1988) 855.
[215] R. Finch, J. Martin, R. Pilkington, J. Antimicrob. Chemother. 22 (1988) 881.
[216] P. Van der Auwera, P. Grenier, Y. Glupeznski, D. Pierard, J. Antimicrob. Chemother. 23 (1989) 209.
[217] P.J. Morrison, T.G. Mant, G.T. Norman, J. Robinson, L. Kunka, Antimicrob. Agents Chemother. 32 (1988) 1503.
[218] C. Siporin, Ann. Rev. Microbiol. 43 (1989) 601.
[219] A.M. Shibl, A.F. Tamfik, S. El-Houfy, F.J. Shammarg, J. Clin. Pharm. Ther. 16 (1991) 353.
[220] G. Carlucci, A. Cilli, M. Liberato, P. Mazzeo, J. Pharm. Biomed. Anal. 11 (1993) 1105.
[221] L. Tan, Y. Yuan, Yaoxue Xuebao 28 (1993) 286.
[222] M. Peng, Yaowu Fenxi Zazhi 15 (1995) 39.
[223] G. Carlucci, P. Mazzeo, G. Palumbo, Chromatographia 43 (1996) 261.
[224] S.R. Knaub, M.F. Chang, C.E. Lunte, E.R. Topp, C.M. Riley, J. Pharm. Biomed. Anal. 14 (1995) 121.
[225] R.T. Foster, R.A. Carr, F.M. Pasutto, J.A. Longstreth, J. Pharm. Biomed. Anal. 13 (1995) 1243.
[226] V. Cecchetti, A. Fravolini, R. Fringuelli, G. Mascellani, P. Pagella, M. Palmiolo, G. Segre, P. Temi, J. Med. Chem. 30 (1987) 465.
[227] R. Mattina, C.E. Cocuzza, M. Cesana, G. Bonfiglio, Chemotherapy 37 (1991) 260.
[228] G. Ravizzola, G. Pinsi, F. Pirali, D. Colombrita, I. Foresti, L. Peroni, A. Turano, Drugs Exp. Clin. Res. 15 (1989) 11.
[229] B.P. Imbimbo, G. Broccali, M. Cesana, F. Crema, G. Attardo-Parrinello, Antimicrob. Agents Chemother. 35 (1991) 390.
[230] J.C. Kisicki, R.S. Griess, L. Ott, G.M. Cohen, R.J. McCormarck, W.M. Troetel, B.P. Imbimbo, Antimicrob. Agents Chemother. 36 (1992) 1296.
[231] R. Mattina, G. Bonfiglio, C.E. Cocuzza, G. Gulisano, M. Cesana, B.P. Imbimbo, Chemotherapy 37 (1991) 389.
[232] R. Wise, J. Johnson, N. O'Sullivan, J.M. Andrews, B.P. Imbimbo, J. Antimicrob. Chemother. 28 (1991) 905.
[233] R. Cogo, R. Mattina, R. Rimoldi, B.P. Imbimbo, Ther. Drug. Monit. 14 (1992) 36.
[234] R. Rimoldi, M. Fioretti, A. Albrici, B.P. Imbimbo, Infection 20 (1992) 89.
[235] J.B.J. Borema, D. Bach, C. Jol, W. Pahlmann, J. Antimicrob. Chemother. 28 (1991) 547.
[236] R. Mattina, C.E. Cocuzza, M. Cesena, Infection 21 (1993) 106.
[237] M. Dirksen, J. Focht, J. Borema, Infection 19 (1991) 297.
[238] G. Carlucci, P. Mazzeo, D. Fanini, G. Palumbo, Ther. Drug Monit. 13 (1991) 448.
[239] T.B. Vree, M. Biggelaar-Martea, A. Peeters, P.B. Imbimbo, J. Chromatogr. 573 (1992) 168.
[240] G. Carlucci, G. Palumbo, J. Chromatogr. 564 (1991) 346.
[241] F. Lombardi, R. Ardemagni, V. Colzani, M. Visconti, J. Chromatogr. 576 (1992) 129.
[242] T.B. Vree, M. van den Biggelaar-Martea, B.P. Imbimbo, Eur. J. Drug Metab. Pharmacokinet. 1 (1992) 45.
[243] G. Segre, D. Cerretani, L. Moltoni, Eur. J. Clin. Pharmacol. 42 (1992) 101.
[244] G. Carlucci, P. Mazzeo, G. Palumbo, Analyst 120 (1995) 2493.
[245] G. Carlucci, P. Mazzeo, J. Chromatogr. Sci. 34 (1996) 182.
[246] S. Hori, J. Shimada, A. Saito, M. Matsuda, T. Mitahara, Rev. Infect. Dis. 11 (1989) 1397.
[247] G. Carlucci, P. Mazzeo, G. Palumbo, J. Chromatogr. 682 (1996) 315.