Analytical Methods for Determining Third and Fourth Generation Fluoroquinolones: A Review

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Abstract  Fluoroquinolones of the third and fourth generation possess wide bactericidal activity. Monitoring concentrations of antibacterial agents provides effective therapy and prevents the increase of bacterial resistance to antibiotics. The pharmacodynamic parameters that best describe fluoroquinolone activity are AUC/MIC and $C_{\text{max}}$/MIC. Determining the level of this type of drug is essential to reach the effective concentration that inhibits the growth of bacteria. Determining the pharmaceutical formulation confirms the purity of a substance. Many methods have been developed to determine the level of these substances. They involve mainly the following analytical techniques: chromatography, capillary electrophoresis, and spectroscopy. The separation techniques were combined with different measuring devices, such as ultraviolet (UV), fluorescence detector (FLD), diode array detector (DAD), and mass spectrometry (MS). The analytical procedures require proper sample pre-conditioning such as protein precipitation, extraction techniques, filtration, or dilution. This paper reviews the reported analytical methods for the determining representatives of the third and fourth generation of fluoroquinolones. Attention was paid to pre-conditioning of the samples and the applied mobile phase. This report might be helpful in the selection of the proper procedure in determining the abovementioned drugs in different matrices.

Keywords  HPLC · UV/Vis spectroscopy · Fluorescence spectroscopy · Mass spectrometry · Pharmacokinetics · Clinical pharmacokinetics

Abbreviations

| Abbreviation | Full Form |
|--------------|-----------|
| ACN          | Acetonitrile |
| ASE          | Accelerated solvent extraction |
| AUC          | Area under the curve |
| BAL          | Bronchoalveolar lavage |
| BALO         | Balofloxacin |
| BCE          | Background electrolyte |
| BCG          | Bromocresol green |
| BPB          | Bromophenol blue |
| $C_{\text{max}}$ | Maximum concentration |
| CAP          | Community acquired pneumonia |
| CE           | Capillary electrophoresis |
| CMPA         | Chiral mobile phase additive |
| DAD          | Diode array detector |
| FLD          | Fluorescence detector |
| GATI         | Gatifloxacin |
| GEMI         | Gemifloxacin |
| HILIC        | Hydrophilic interaction liquid chromatography |
| HPLC         | High performance liquid chromatography |
| HPTLC        | High performance thin layer chromatography |
| LEVO         | Levofloxacin |
| LLE          | Liquid–liquid extraction |
| MBTH         | 3-Methyl-2-benzothiazolinone hydrazone hydrochloride |
| MDR-TB       | Multidrug-resistant tuberculosis |
| MIC          | Minimum inhibitory concentration |
| MOXI         | Moxifloxacin |
| MRM          | Multiple reaction mode |
| MS           | Mass spectrometry |
| NMR          | Nuclear magnetic resonance |
| PAZU         | Pazufloxacin |

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Introduction

Fluoroquinolones are a vast class of synthetic bactericidal agents widely used in treatment. In 1963 nalidixic acid was the first quinolone approved by FDA. The intensive development of this group of antibacterial drugs was in the 1980s with the discovery that a combination of fluorine atoms at position 6 and a piperazinyl group at position 7 of the quinoline ring expands the spectrum of bactericidal activity. This modification in structure produced norfloxacin, the first quinolone approved by FDA. The intensive development of this group of antibacterial drugs was in the 1980s. The third generation representatives are levofloxacin (LEVO), balofloxacin (BALO), pazufloxacin (PAZU), and sparfloxacin (SPAR) [12]. LEVO is used in the treatment of the community-acquired pneumonia (CAP), acute maxillary sinusitis, and acute exacerbation of chronic bronchitis. LEVO is also used in the eradication of *Helicobacter pylori* when standard therapies fail. The oral and intravenous administration of LEVO are equivalent due to its full bioavailability. It is poorly metabolized—after 48 h about 87% of unchanged drug is eliminated in urine. The main metabolites are N-oxide and desmethyllevofloxacin, and they are inactive [12–14]. BALO exhibits excellent antibacterial activity against Gram-positive bacteria such as multi-drug-resistant *Staphylococci* and *Pneumococci*. It is metabolized in the kidneys to glucuronide and N-desmethyl derivative [15]. PAZU has strong activity against Gram-negative bacteria, and it easily permeates the liver tissue, gallbladder tissue, and bile. This indicates that PAZU might be useful in the treatment of patients with the liver disease [16]. SPAR is reported to be more active in vitro than ciprofloxacin against *Mycobacteria* and Gram-positive bacteria, as well as *Mycoplasma*, *Chlamydia*, and *Legionella*. Their activity is based on inhibition of bacterial enzymes: DNA gyrase and DNA topoisomerase IV. These enzymes are necessary to separate bacterial DNA. This activity leads to inhibition of cell replication [2–6].

The activity of fluoroquinolones strongly depends on their concentration. Pharmacokinetic parameters may exhibit interpatient variability, especially in some groups of patients, such as the critically ill, those with renal impairment, or hospitalized patients. Pharmacodynamic parameters that best describe the efficacy are the area under the plasma concentration–time curve to minimum inhibitory concentration (AUC/MIC) and maximum plasma drug concentration to minimum inhibitory concentration ($C_{\text{max}}$/MIC). The optimal value of these parameters provides the effective pharmacotherapy of bacterial diseases and thus prevents bacterial resistance and lack of therapy efficacy [7–11].

In the analysis of fluoroquinolones many high performance liquid chromatography (HPLC) methods with different detection techniques were applied. The most common is HPLC with ultraviolet (HPLC-UV) or fluorescence detection (HPLC-FLD). Another detector combined with the HPLC system is mass spectrometry (MS) (HPLC–MS). These methods are common for determining drugs in serum; however, mass detection makes it possible to determine very low concentration in matrix. Another separation technique for analysis of the fluoroquinolone levels is capillary electrophoresis (CE) combined with UV or FLD. The determination of drug level in pharmaceutical formulation can be performed by both HPLC techniques and others such as UV-spectroscopy, voltamperometry, or even nuclear magnetic resonance (NMR). All the aforementioned methods require proper preparation of the sample. The pharmaceutical formulations are the least complex matrices—only dilution is required. Physiological fluids (blood, bile, saliva, and urine) and tissues homogenates require a more complex technique of separation. This is due to the presence of endogenous substances that may appear on the chromatogram or electropherogram during analysis. The key factor is to optimize the conditions of the analysis (use of the proper solvent or buffer) and the sample preparation. In this case the sample preparation may involve dilution which might be applied for urine, protein precipitation or extraction applied in more complex matrices (blood, serum, tissue homogenates). This paper reports information about the methods for determining representatives of the third and fourth generation of fluoroquinolones in different matrices. The methods are divided according to the used analytical technique used and preconditioning of samples for analysis.

Third Generation Fluoroquinolones

The third generation representatives are levofloxacin (LEVO), balofloxacin (BALO), pazufloxacin (PAZU), and sparfloxacin (SPAR) [12]. LEVO is used in the treatment of the community-acquired pneumonia (CAP), acute maxillary sinusitis, and acute exacerbation of chronic bronchitis. LEVO is also used in the eradication of *Helicobacter pylori* when standard therapies fail. The oral and intravenous administration of LEVO are equivalent due to its full bioavailability. It is poorly metabolized—after 48 h about 87% of unchanged drug is eliminated in urine. The main metabolites are N-oxide and desmethyllevofloxacin, and they are inactive [12–14]. BALO exhibits excellent antibacterial activity against Gram-positive bacteria such as multi-drug-resistant *Staphylococci* and *Pneumococci*. It is metabolized in the kidneys to glucuronide and N-desmethyl derivative [15]. PAZU has strong activity against Gram-negative bacteria, and it easily permeates the liver tissue, gallbladder tissue, and bile. This indicates that PAZU might be useful in the treatment of patients with the liver disease [16]. SPAR is reported to be more active in vitro than ciprofloxacin against *Mycobacteria* and Gram-positive bacteria, as well as *Mycoplasma*, *Chlamydia*, and *Legionella*. Their activity is based on inhibition of bacterial enzymes: DNA gyrase and DNA topoisomerase IV. These enzymes are necessary to separate bacterial DNA. This activity leads to inhibition of cell replication [2–6].

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bacteria including *Streptococcus pneumoniae* and other *Streptococci* and *Staphylococci* [17].

**Levofloxacin**

There are many analytical techniques for quantitative analysis of this drug in different matrices (Table 1). Most of them are based on reversed phase HPLC. These techniques are well suited due to the solubility of LEVO in water. The most common applied detectors are UV [18–25] and FLD [20, 26–31]. However, if the lower level of quantification is required, mass detection (MS) can be applied [32–35]. In MS/MS analysis the following multiple reaction modes (MRM) are employed: 

\[
\frac{m}{z} 362.7 \rightarrow 261.2 [32], \frac{m}{z} 362.1 \rightarrow 318.1 [34], \text{ and } \frac{m}{z} 362.2 \rightarrow 261.2 [35].
\]

For single MS the following selected reaction monitoring is observed 

\[
\frac{m}{z} 362 \rightarrow 318 [33].
\]

The other detector that can be applied is photodiode array detector PDA [36]. The mobile phase is a mixture of water or aqueous buffer and organic solvent. Triethylamine (TEA) is used as an addition to mobile phase. TEA is an ion pair reagent added to water that improves the shape of the peak. Its content does not exceed 1%, and the pH of the mobile phase is slightly acidic [19, 21, 25, 26, 31, 36]. The proper pH value is shifted with orthophosphoric acid. The addition of ion pair reagent improves the quality of the separation due to the presence of the negatively charged carboxyl group. The other polar constituent might be the phosphate buffer consisting of either sodium or potassium phosphates in the following range of concentrations 10–30 mM [19, 24, 29, 36]. The most common organic solvent in HPLC separation is acetonitrile (ACN) [20–22, 25–27, 29–31, 33, 35, 36]. Its content is within the range 14–43% for isocratic elution [20–22, 25, 26, 31, 36] and is also applied in gradient elution; however, in this case the content of ACN varies in time [27, 33, 35]. The high content of ACN is characteristic for separation on a hydrophilic interaction liquid chromatography (HILIC) column where the content of organic solvent is higher than 80% [32].

The pre-dominant type of chromatographic column used for RP-HPLC analysis is C18; however, there are other columns, e.g. C8 or C4, on which the separation is performed (Table 1). Watabe et al. tested different types of columns, e.g. C18 and C8, in LEVO and also pazufloxacin (PAZU) analysis. It was mentioned that LEVO and PAZU interact better with the C8 column because this column possesses less steric hindrance than the C18 column. The structure of these substances differs in the C-10 position of 7-oxopyrido[1,2,3-de] benzoxazine-6-carboxylic acid. LEVO and PAZU posses a 4-methylpiperezinyl group and 1-aminocyclopropyl group, respectively. The presence of these groups may cause a better interaction with the surface of the stationary phase. Fang et al. used the C4 column in the separation. In this analysis, besides LEVO, also isoniazid and rifampicin were detected. The analysed compounds were in a wide range of polarity, and this type of column was more suitable than C18. The butyl bonded stationary phase provides a shorter time of analysis of non-polar compounds without significantly affecting the separation of the polar ones. The high resolution is still maintained when compared with a long chain bonded stationary phase. HILIC columns were also applied in LEVO analysis. The main advantage to using HILIC columns is the fact that they can be used for separation of ionized compounds. The HILIC columns are suitable for MS detection due to the high content of organic solvent. HILIC separation is a normal type of separation, but the typical reversed phase eluents are used. It is helpful when the poor retention of the analyte is observed in the column [26, 32, 34]. Methanol is often used in addition to ACN, and it can be used in both isocratic [23] and gradient elution [19, 23, 27, 28]. ACN, water, and methanol (and their mixtures) might be used as the solvents for stock solutions [37]. The other contents of the mobile phase might be chiral mobile phase additive (CMPA) solution consisting of CuSO4 and l-leucine [18], formic acid (in MS detection) [33, 35], sodium dodecylsulfate (SDS) [20], tetrabutylammonium acetate (TBA) [20], citric acid [20, 22], ammonium acetate [22, 35], tetrabutylammonium bromide (TBA) [29], and l-isoleucine [23]. Liang et al. [20] reported the use of SDS in mobile phase as an agent that increases the retention time not only for LEVO, but also for gatifloxacin (GATI), moxifloxacin (MOXI), and trovafloxacin (TROVA). It was used in addition to 25 mM phosphate buffer and ion pair reagent (10 mM TBA), which improved the shape of the peaks. This composition of the mobile phase makes it possible to overcome the secondary interactions between silanol groups on the stationary phase and amino groups on quinolones. The addition of CuSO4, l-leucine or l-isoleucine enables the stereospecific determination of LEVO in matrix. Stereoselectivity was achieved through incorporation of chiral ligand exchange reagents directly into mobile phase. The Cu2+ ions, l-leucine, and water form a complex that combines with LEVO and its R-enantiomer. These complexes have different configurations. They might be applied for the determination of impurities in pharmaceutical formulations. The other aminooacids were tested (l-phenylalanine, l-serine and l-alanine); however, the best resolution was observed for l-leucine [18]. Devi et al. reported also the method for determination of impurities after oxidative degradation; however, it was not stereospecific [19].

The next separation technique that might be applied for LEVO analysis is CE. This method requires a relatively small amount of analyte. It may be applied for quantification of LEVO in different matrices such as human urine, tablets, or in water. The separation might be performed in...
| Analysed compound | Sample | Method | Sample preparation | LOD/LOQ | References |
|-------------------|--------|--------|--------------------|---------|------------|
| LEVO              | Pure form, pharmaceutical formulation | VIS-spectroscopy, $\lambda = 424$ nm for LEVO-BPB and $\lambda = 428$ nm for LEVO-BCG | LLE with chloroform | LLOQ for LEVO-BCG and LEVO-BPB—1.85 µg mL$^{-1}$ | [41] |
| LEVO              | Pharmaceutical formulation | HPLC-UV, chiral separation with mixture of L-leucine and Cu$^{2+}$ ions (CMPA), column: C18 OptimaPAk 150 mm × 4.6 mm, 5 µm, mobile phase: CMPA solution—MeOH (88:12), isocratic elution | Not reported | LLOQ—0.5 mg L$^{-1}$ | [18] |
| LEVO              | Urine | Adsorptive square-wave anodic stripping voltammetry | Dilution with acetate buffer | LOD—10 µg mL$^{-1}$ | [44] |
| LEVO              | Sputum | MEPS–HPLC–PDA, column: C8 discovery column (250 mm × 4.6 mm, 5 µm), mobile phase: phosphate buffer (30 mM, pH 2.5, 1% TEA) and ACN (1% TEA) 86:14 (v/v) isocratic elution | MEPS extraction | LOQ—0.05 µg mL$^{-1}$ | [36] |
| LEVO              | Tablets, urine and serum | Fluorescent spectroscopy, $\lambda_{excitation} = 292$ nm, $\lambda_{emission} = 494$ nm, the fluorescence was enhanced by micelle | Tablets were powdered and dissolved in an ethanol–water mixture; urine was dissolved in water; serum did not require pre-treatment | LOD—10 ng mL$^{-1}$ | [43] |
| LEVO              | Pharmaceutical formulation, urine, plasma | $^1$H NMR spectroscopy | Tablets were powdered and dissolved in borate buffer; urine and plasma samples were diluted with D$_2$O | Pharmaceutical formulation: LOD—0.134 mg·0.6 mL$^{-1}$, LOQ—0.446 mg·0.6 mL$^{-1}$; Urine: LOD 0.015 mg·0.6 mL$^{-1}$, LOQ—0.05 mg·0.6 mL$^{-1}$; Plasma: LOD—0.153 mg·0.6 mL$^{-1}$, LOQ—0.5 mg·0.6 mL$^{-1}$ | [45] |
| LEVO              | Pharmaceutical formulation | Synchronous scanning room temperature phosphorimetry | Boiling in a Soxhlet apparatus | ALOD—13 ng, ALOQ—41 ng | [46] |
| LEVO              | Plasma | HILIC–MS/MS, column: HILIC Silica Column (50 mm × 3.0 mm, 5 µm), mobile phase: ACN—ammonium formate (100 mM pH 6.5) 82:18 (v/v), isocratic elution, temperature 30 °C | LLE with dichloromethane | LLOQ—10 ng mL$^{-1}$ | [32] |
| LEVO              | Tissue homogenate | LC–MS, column: Symmetry C18 (100 mm × 2.1 mm, 3.5 µm), mobile phase: A: ACN, water, formic acid (3:97:0.2 v/v/v), B: ACN: formic acid (100:0.2 v/v), gradient elution | SPE | LOQ—0.02 µg g$^{-1}$ | [33] |
| LEVO              | Reaction mixture | RP–HPLC–UV, column: ACE C18 column (250 mm × 4.6 mm, 5 µm), mobile phase: A: 25 mM NaH$_2$PO$_4$, 0.5% TEA pH 6.0, B: methanol, gradient elution, temperature 40 °C | Dilution of the samples with a mixture of water:ACN (60:40) | Not reported | [19] |
| Analysed compound | Sample | Method | Sample preparation | LOD/LOQ | References |
|-------------------|--------|--------|--------------------|---------|------------|
| LEVO              | Plasma, bone tissue, BAL | HPLC–UV, column: ABZ + Supelcosil (150 mm × 4.6 mm, 5 μm), mobile phase: 0.4% TEA pH 3.0: ACN (83:17 v/v), isocratic elution | SPE | LOD—0.05 μg g⁻¹ (plasma), 0.1 μg g⁻¹ (BAL), 0.2 μg g⁻¹ (bone), LOQ—0.2 μg g⁻¹ (plasma), 0.4 μg g⁻¹ (BAL), 0.5 μg g⁻¹ (bone) | [21] |
| LEVO              | Tissues, plasma | HPLC–MS/MS, column: C4 Welsch Materials (250 mm × 4.6 mm, 5 μm), mobile phase: A: 0.05% formic acid, B: methanol, gradient elution | Protein precipitation with methanol | LOD—0.05 μg g⁻¹ (tissues), 6.6 ng mL⁻¹ (plasma), LLOQ—0.13 μg g⁻¹ (tissues), 21.8 ng mL⁻¹ (plasma) | [34] |
| LEVO              | Bulk and marketed formulations | UV spectroscopy, λ = 292 nm | Sample dilution | LOD—0.021 μg mL⁻¹, LOQ—0.064 μg mL⁻¹ | [42] |
| LEVO              | Tablets | HPLC–UV, column: C18 Cosmosil MS II, mobile phase: 0.05 M citric acid monohydrate: 1 M ammonium acetate: ACN (84:1:15), isocratic elution | Tablets were pulverized, dissolved, and diluted | Not reported | [22] |
| LEVO              | Plasma, dialysate | HPLC–FLD, column: YMC PRO C18 (150 mm × 2 mm, 5 μm), mobile phase: A: (MeOH: 1.0 M NH₄OAc:H₂O—10:5:85 v/v/v), B: (MeOH: 1.0 M NH₄OAc:H₂O—40:5:55 v/v/v), gradient elution | Protein precipitation with 50% TFA | LLOQ—0.1 μg mL⁻¹ | [28] |
| LEVO              | Plasma, urine | HPLC–UV, column: C18 Intersil ODS-2 (250 mm × 4.6 mm, 5 μm), mobile phase: (5 mM CuSO₄, 10 mM l-isoleucine):methanol 87.5:12.5, isocratic elution, temperature 35 °C | LLE with dichloromethane | LLOQ—0.08 μg mL⁻¹ (plasma), 23 μg mL⁻¹ (urine) | [23] |
| LEVO              | Blood, bile | HPLC–FLD, column: C18 LiChrospher (250 mm × 4.6 mm, 5 μm), mobile phase: ACN: 1 mM 1-octanesulfonic acid (40:60 v/v, pH 3.0), isocratic elution | Microdialysis | LOD—50 ng mL⁻¹, LLOQ—0.1 μg mL⁻¹ | [30] |
| LEVO              | Plasma | HPLC–FLD, column: C18 LiChroCART (125 mm × 4 mm), mobile phase: ACN—0.4% TEA pH 3.0 (24:76 v/v), isocratic elution | Protein precipitation with ACN | LOD 0.03 mg L⁻¹, LLOQ 0.15 mg L⁻¹ | [31] |
| LEVO              | Urine | CE with electroluminescence detection, capillary: a fused-silica capillary 55 cm × 50 μm i.d. × 375 μm o.d., 18 kV, buffer: 20 mM PBS pH 8.0 | Precipitation with ACN | LOD—6.4 × 10⁻⁷ M, LOQ—1.4 × 10⁻⁸ M | [38] |
| LEVO              | Water | CE with UV detection, capillary: fused silica capillary 80 cm × 75 μm i.d., BGE (3 M acetic acid, 49 mM ammonium acetate in 55:45 v/v methanol: ACN), 30 kV, temperature 20 °C | Dispersive liquid–liquid microextraction | LOD—5.74 μg mL⁻¹, LOQ—19.1 μg mL⁻¹ | [40] |
| LEVO/PAZU         | Serum | HPLC–FLD, column: Intersil C8-3 (250 mm × 4.6 mm, 5 μm), mobile phase: 1% TEA pH 3.0: ACN (86:14 v/v), isocratic elution | Protein precipitation with 6% HClO₄ and methanol | LLOQ—0.1 μg mL⁻¹ (LEVO and PAZU) | [26] |
| Analysed compound | Sample | Method | Sample preparation | LOD/LOQ | References |
|-------------------|--------|--------|--------------------|---------|------------|
| LEVO/MOXI/GATI    | Tablets| CE with UV detection, capillary: a fused-silica capillary 48.5 cm × 50 μm i.d. × 375 μm o.d., 25 kV; buffer: 25 mM TRIS/hydrochloride and 15 mM sodium tetraborate pH 8.87, temperature 25 °C | Tablets were powdered and dissolved | LOD—1.02 mg L⁻¹, LOQ—3.40 mg L⁻¹ (LEVO), LOD—1.53 mg L⁻¹, LOQ—5.11 mg L⁻¹ (MOXI), LOD—1.51 mg L⁻¹, LOQ—5.03 mg L⁻¹ (GATI) | [39] |
| LEVO/MOXI/GATI/TROVA | Plasma | HPLC–UV/FLD, column: Adsorbsphere C18 (250 mm × 4.6 mm, 5 μm), mobile phase: A: 10 mM SDS, 10 mM TBA, 25 mM citric acid, B: ACN pH 3.5 (57:43 v/v), isocratic elution | Ultrafiltration of pre-conditioned sample with SDS 0.5% solution | LLOQ—50 ng mL⁻¹ for UV detection, and 20 ng mL⁻¹ for FLD detection (LEVO, MOXI, GATI, TROVA) | [20] |
| LEVO/MOXI/PAZU/GATI/TROVA | Plasma | HPLC-FLD, column: C18 LiChroCART Purospher Star (55 mm × 4.0 mm, 3 μm), mobile phase: A: 0.1% formic acid adjusted to pH 3.0 with TEA, B: ACN and C: MeOH, gradient elution | Protein precipitation with ACN | LOD—0.01 μg mL⁻¹ (LEVO, PAZU, MOXI), 0.02 μg mL⁻¹ (TROVA), 0.0025 μg mL⁻¹ (GATI); LOQ—0.02 μg mL⁻¹ (LEVO, PAZU, MOXI), 0.04 μg mL⁻¹ (TROVA), 0.005 μg mL⁻¹ (GATI) | [27] |
| LEVO/MOXI/GATI | Serum | HPLC-FLD with column switching, ABZ + Plus Supelcosil (150 mm × 4.6 mm, 5 μm), mobile phase: I. (10 mM K₂HPO₄ pH 5.4: ACN 98:2 v/v), II. (10 mM KH₂PO₄ pH 2.5 with 2 mM TBAmBr: ACN 88:12 v/v), isocratic elution | Online extraction on pre-column | LOD—60 ng mL⁻¹, LOQ—125 ng mL⁻¹ (LEVO) LOD—35 ng mL⁻¹, 125 ng mL⁻¹ (MOXI), LOD—120 ng mL⁻¹, LOQ—162.5 ng mL⁻¹ (GATI) | [29] |
| LEVO/MOXI/GATI | Plasma | HPLC–UV, column: Hypersil BDS C18 column (250 mm × 4.6 mm, 5 μm), mobile phase: 20 mM NaH₂PO₄ pH 3.2: ACN (75:25 v/v), isocratic elution | Protein precipitation with ACN: methanol mixture (1:1) | LLOQ—0.1 μg mL⁻¹ (LEVO, MOXI and GATI) | [24] |
| LEVO/GATI | Pharmaceutical formulations | HPLC–UV, column: C18 LiChropher 100 (125 mm × 4.0 mm, 5 μm), mobile phase: water and ACN (80:20 v/v) with addition of 0.3% TEA, pH 3.3, isocratic elution | Tablets were powdered and dissolved and ampoules were diluted | LLOQ—4 μg mL⁻¹ | [25] |
| LEVO/SPAR | Chicken breast muscle | HPLC–MS/MS, column: C18 Symmetry (150 mm × 4.6 mm, 5 μm), mobile phase: A:54 mM formic acid 10 mM CH₃COONH₄, B: ACN, gradient elution, temperature 30 °C | Extraction with ACN with 0.3% addition of phosphoric acid (v/v)water (70:30) and hexane | LOD—3.6 ng g⁻¹, LLOQ—11.9 ng g⁻¹ (LEVO) LOD—2.7 ng g⁻¹, LLOQ—8.9 ng g⁻¹ (SPAR) | [35] |
| BALO | Plasma | HPLC–MS, column: C18 Agilent ZORBAX 300SB (150 mm × 2.1 mm), mobile phase: methanol: water (10 mM CH₃COONH₄, pH 3.0) (40:60 v/v), isocratic elution, temperature 40 °C | LLE with the mixture dichloromethane and ethyl acetate (20:80 v/v) | LOD—0.02 μg mL⁻¹, LLOQ—0.03 μg mL⁻¹ | [15] |
| BALO | Tablets | HPLC–UV, column: C18 Zodiac (150 mm × 4.6 mm, 5 μm), mobile phase: 0.01 M KH₂PO₄; ACN pH 6.5, (40:60 v/v), isocratic elution, temperature 30 °C | Sample dilution | LOD—0.85 μg mL⁻¹, LOQ—2.58 μg mL⁻¹ | [48] |
**Table 1 continued**

| Analysed compound | Sample | Method | Sample preparation | LOD/LOQ | References |
|-------------------|--------|--------|--------------------|---------|------------|
| **PAZU**          | Saliva, gingival crevicular fluid, serum | HPLC–UV, C18 Agilent ZORBAX SB (250 mm × 4.6 mm, 5 μm), mobile phase: ACN: 0.5% phosphoric acid containing 1% TEA (155:850 v/v), isocratic elution | Protein precipitation with methanol | LOD—10 ng mL⁻¹ | [49] |
| **PAZU**          | Tablets, milk | CE with potential gradient detection, capillary: fused-silica capillary (8.5 cm × 75 μm i.d. × 375 μm o.d., 3 kV, buffer: 30 mM Tris and 4 mM phosphoric acid at pH 8.9, temperature 20 °C | LLE with dichloromethane | LOD—39 ng mL⁻¹, LOQ—130 ng mL⁻¹ | [50] |
| **PAZU**          | Muscle | CE with potential gradient detection, capillary: fused-silica capillary (7.8 cm × 50 μm i.d., 3 kV, buffer: 30 mM Tris and 3 mM phosphoric acid at pH 9.0, temperature: ambient | LLE with dichloromethane | LOD—0.3 mg L⁻¹ | [51] |
| **PAZU/GATI**     | Urine | CE with UV detection, capillary: fused-silica capillary (50 cm × 50 μm i.d. × 375 μm o.d., 10 kV, buffer: 70 mM phosphate buffer and 40 mM hydroxypropylβ-cyclodextrin at pH 5.04 for pazufl oxacin and 20 mM hydroxypropyl β-cyclodextrin at pH 3.90 for gatifloxacin, temperature: 20 °C | Dilution with water | LOD—7 μg mL⁻¹ for PAZU | [52] |
| **SPAR**          | Pharmaceutical formulations | UPLC-UV, column: C18 Waters Acquity HSS T-3 column (100 mm × 2.1 mm, 1.8 μm), mobile phase: A: 0.1% orthophosphoric acid, B: ACN, gradient elution, temperature 50 °C | Tablets were pulverized and dissolved, eye drops were dissolved | LOD—0.2 μg mL⁻¹, LOQ—0.6 μg mL⁻¹ | [17] |
| **SPAR**          | Plasma, urine | HPLC–UV, column: C18 YMC pack A-312 (150 mm × 6 mm, 5 μm), mobile phase: 5% acetic acid:ACN: methanol (76:12:12) with 1% addition of TEA to mobile phase, isocratic elution, temperature 50 °C | Protein precipitation with 20% perchloric acid for plasma samples. The urine samples were diluted with water | LOQ—0.025 mg L⁻¹ (plasma), 0.5 mg L⁻¹ (urine) | [53] |
| **SPAR/SPAR**     | Plasma | HPLC–UV, column: C18 Kromasil 100 (250 mm × 4.6 mm, 5 μm), mobile phase: NaH₂PO₄ (pH 2.5): ACN (80:20 v/v), isocratic elution, temperature 35 °C | LLE with ethyl acetate | LOQ—100 ng mL⁻¹ for each compound | [54] |
| **SPAR**          | Plasma | HPLC–MS/MS, column: C18 Atlantis (50 mm × 2.1 mm, 3 μm), mobile phase: 10 mM CH₃COONH₄ (pH 4.0): ACN (20:80 v/v), isocratic elution | Protein precipitation with ACN | LLOQ—10 ng mL⁻¹ | [55] |
| **SPAR/PAZU/GATI**| Serum | CE with UV detection, capillary: fused-silica capillary (65 cm × 50 μm i.d. × 375 μm o.d., 10 kV, buffer: 12 mM disodium tetraborate pH 9.08 in addition of silica nanoparticles (5.2 μg/mL), temperature: 25 °C | Dilution with buffer solution | LOD—2 μg mL⁻¹ (for GATI, PAZU), 2.5 μg mL⁻¹ (SPAR), LLOQ—5 μg mL⁻¹ (for GATI, SPAR), 6 μg mL⁻¹ (PAZU) | [56] |
| **MOXI**          | Pure substance, tablets | Vis-spectroscopy, λ = 623 nm (complexes with MBTH in the presence with Ce IV ions), λₘₓ = 623 nm | Tablets were pulverized and dissolved | LOD—0.043 μg mL⁻¹, LOQ—1.89 μg mL⁻¹ | [85] |
| Analysed compound | Sample | Method | Sample preparation | LOD/LOQ | References |
|-------------------|--------|--------|--------------------|---------|------------|
| MOXI              | Eye drops | HPLC–UV–DAD, column: C8 BDS Hypersil column (250 mm × 4.6 mm, 5 µm), mobile phase: 20 mM phosphate buffer with 0.1% TEA pH 2.8: methanol (38.5:61.5 v/v), isocratic elution | Eye drops were diluted with mobile phase | LOD—0.316 µg mL⁻¹, LOQ—1.014 µg mL⁻¹ | [66] |
| MOXI              | Plasma | LC–MS/MS, column: ODS C18 (150 mm × 4.6 mm, 5 µm); mobile phase: methanol: 0.03% TEA (85:15 v/v), isocratic elution, temperature 30 °C | Protein precipitation with methanol | LLOQ—0.1 µg mL⁻¹ | [80] |
| MOXI              | Eye drops | RP–HPLC–UV, column: Grace Smart C18 (250 mm × 4.6 mm, 5 µm), mobile phase: 25 mM KH₂PO₄ with addition of 0.5% TEA pH 3.2 (60:40 v/v), isocratic elution | Eye drops diluted with mobile phase | LOD—0.098 µg mL⁻¹, LOQ—0.327 µg mL⁻¹ | [74] |
| MOXI              | Saliva | HPLC-FLD, column: C18 Lichrospher 100 RP-18e (150 mm × 4.6 mm, 5 µm), mobile phase: 50 mM phosphate buffer (KH₂PO₄) pH 2.6: ACN (80:20 v/v), isocratic elution | Protein precipitation with 7% HClO₄ | LOD—0.03 µg mL⁻¹, LOQ—0.1 µg mL⁻¹ | [76] |
| MOXI              | Blood | LC–MS/MS, column: C18 Hy-Purity (50 mm × 2.1 mm, 5 µm), mobile phase: A: (ammonium acetate 10 g/L, acetic acid 35 mg/L, trifluoroacetic anhydride 2 mL/L water), B: water, C: ACN, gradient elution | Extraction with cyanomipramine in mixture of methanol and water | LLOQ—0.05 mg L⁻¹ | [81] |
| MOXI              | Pharmaceutical formulations | HPLC–UV, column: Waters Xterra C18 Purity (50 mm × 2.1 mm, 5 µm), mobile phase 2% TEA pH 6.0 and ACN (90:10 v/v), isocratic elution | Formulations were powdered and dissolved | LOD—0.05 µg mL⁻¹, LOQ—0.2 µg mL⁻¹ | [60] |
| MOXI              | Plasma | HPLC–UV, column: C8 Kromasil (250 mm × 4.6 mm, 5 µm), mobile phase: ACN: methanol: 20 mM TEA pH 3.0 KH₂PO₄ (15:20:65 v/v/v) at 30 °C isocratic elution | LLE with dichloromethane | LOD—0.015 µg mL⁻¹, LLOQ—0.05 µg mL⁻¹ | [75] |
| MOXI              | Plasma | LC ESI–MS/MS, column: BDS Hypersil C18 (100 mm × 4.6 mm, 5 µm), mobile phase: 0.1% formic acid and ACN (60:40 v/v) | SPE | LOD—50 pg mL⁻¹, LOQ—1 ng mL⁻¹ | [82] |
| MOXI              | Vitreous and aqueous humor | HPLC–FLD, ACCQ Taq aminoacid analysis column (150 mm × 3.9 mm, 4 µm) mobile phase, A: ACN, methanol, 0.05 M TBA·Cl, TFA (37.5:12.5:949:1 v/v/v/v), B: ACN: methanol, 0.05 M TBA·Cl, TFA (75:25:899:1v/v/v/v) pH 3.0, gradient elution, temperature 30 °C | Dilution with mobile phase for aqueous humor and protein precipitation with ACN for vitreous humor | LOD—10 ng mL⁻¹ | [77] |
| MOXI              | Plasma | HPLC–FLD, column: supelco LC-Hisep column (150 mm × 4.6 mm, 5 µm), mobile phase: ACN: 0.25 M Na₂PO₄ pH 3.0 (5:95 v/v), isocratic elution | Microfiltration in syringe | Plasma: LOD—1 µg L⁻¹, LOQ—3 µg L⁻¹, Water: LOD—0.1 µg L⁻¹, LOQ—1 µg L⁻¹ | [78] |
| MOXI/SPAR         | Plasma | HPLC–DAD/FLD, column: Altantis dC18 column (150 mm × 4.6 mm, 5 µm), mobile phase: ACN and 0.1% TFA, gradient elution | Protein precipitation with ACN | LOD—0.04 µg mL⁻¹ (MOXI) FLD detection, 0.3 µg mL⁻¹ (SPAR) DAD detection | [57] |
| Analysed compound | Sample | Method | Sample preparation | LOD/LOQ | References |
|-------------------|--------|--------|--------------------|---------|------------|
| MOXI/GATI | Muscle | CE with capacitively coupled contactless conductivity detection, capillary: fused-silica capillary (42 cm × 50 μm i.d. × 375 μm o.d.) 13 kV, buffer: BGE 85% (10 mM tartaric acid, 14 mM sodium acetate) and 15% methanol (v/v) pH 3.8, room temperature | LLE with dichloromethane, the homogenate was defatted with n-hexane | LOD—0.33 μg mL⁻¹ (MOXI), 0.45 μg mL⁻¹ (GATI), LOQ—1.4 μg mL⁻¹ (MOXI), 2.1 μg mL⁻¹ (GATI) | [83] |
| MOXI | Drug substance and ophthalmic drug | CE–DAD, capillary (40 cm × 50 μm i.d.) 13 kV, buffer: BGE (12.5 mM TEA pH 2.5 with 5% highly sulfated γ-cyclodextrin and 6% ACN), temperature 20 °C | Sample dilution | LOQ—0.055 μg mL⁻¹ | [84] |
| MOXI/GATI | Aqueous humor and vitreous humor | HPLC–PAD/FLD, column: LiChrospher RP-18 (125 mm × 4 mm, 5 μm), mobile phase: ACN—0.1% TFA (pH 3.0) with 30 mM TBA·Cl (20:80), isocratic elution | Aqueous humor samples were diluted, vitreous humor samples were extracted by SPE | LLOQ—0.01 μg mL⁻¹ (MOXI and GATI) | [79] |
| TROVA | Plasma | HPLC–UV, column: C18 Phenomenex Luna (150 mm × 4.6 mm, 5 μm), mobile phase: A: 17.5 mM NaH₂PO₄, 1.5 mM tetrabutylammonium hydroxide pH 3.0, B: ACN and methanol (1:1 v/v), gradient elution | Protein precipitation with 20% HClO₄ | LOD—2 ng mL⁻¹ | [67] |
| TROVA | Serum, urine | HPLC–FLD, column: for serum C18 Nucleosil 100-5 (125 mm × 4 mm, 5 μm) and for urine C18 Nucleosil SA (125 × 4 mm, 5 μm), mobile phase: for serum ACN and 14.3 mM tetrabutylammonium hydrogensulfate: ACN—concentrated phosphoric acid: water (700:250:1.5:48.5 v/v) pH 3.68, for urine: acetonitrile: sodium phosphate solution (622:378) pH 3.60 isocratic elution | Protein precipitation with a mixture of ACN and HClO₄ (99.75: 0.25) for serum, Dilution with sodium phosphate solution pH 3.6 for urine | LOD—0.02 mg L⁻¹ (serum), 0.1 mg L⁻¹ (urine), LLOQ—0.07 mg L⁻¹ (serum), 0.5 mg L⁻¹ (urine) | [86] |
| TROVA | Serum, urine | Differential-pulse adsorptive stripping voltammetry | Filtration | LOD—0.6 ng mL⁻¹, LOQ—2 ng mL⁻¹ | [68] |
| SITA | Urine | HPLC–MS/MS, column: C18 Agilent Proshell 120-SB (50 mm × 2.1 mm, 2.7 μm), mobile phase: methanol: 0.1% formic acid solution (38:62 v/v), isocratic elution, temperature 40 °C | Protein precipitation in 0.1% formic acid methanol solution | LLOQ—0.025 μg mL⁻¹ | [87] |
| SITA | Plasma | HPLC–MS/MS, column: C18 ZORBAX SB (100 mm × 2.1 mm, 3.5 μm), mobile phase: methanol: 0.1% formic acid (46:54 v/v), isocratic elution, temperature 35 °C | Protein precipitation with isopropanol | LLOQ—5 ng mL⁻¹ | [69] |
| PRULI | Tablets | HPLC–UV, column: C18 Sunfire (250 mm × 4.6 mm, 5 μm), mobile phase: ACN: KH₂PO₄ buffer pH 7.30 adjusted with TEA (10:90 v/v), isocratic elution | Tablets were pulverized and dissolved in mobile phase | LOD—0.1404 μg mL⁻¹, LOQ—0.4255 μg mL⁻¹ | [70] |
Table 1 continued

| Analysed compound | Sample                  | Method                                                                 | Sample preparation                                                                 | LOD/LOQ                      | References |
|-------------------|-------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------------------|------------------------------|------------|
| PRULI (ULI)       | Tablets                 | HPLC–UV, column: LUNA HILIC (250 mm × 4.6 mm, 5 µm), mobile phase: ACN: ammonium acetate (5 mM, pH 5.8) (88:12 v/v), isocratic elution | Tablets were pulverized and dissolved in methanol                                  | LOD—0.15 µg mL⁻¹ (PRULI), 3.0 µg mL⁻¹ (ULI) LLOQ—0.25 µg mL⁻¹ (PRULI), 5.0 µg mL⁻¹ (ULI) | [88]       |
| PRULI (ULI)       | Aqueous human humor     | HPLC–UV, column: C8 ZORBAX ECLIPSE XDB (150 mm × 4.6 mm, 5 µm), mobile phase: ACN and 85% aqueous phosphoric acid (15:85 v/v), isocratic elution | Dilution with mobile phase                                                        | LOD—5 ng mL⁻¹ (ULI), LLOQ—6 ng mL⁻¹ (ULI) | [89]       |
| PRULI              | Degradation products    | HPLC MS/MS, column: C18 Waters symmetry (250 mm × 4.6 mm, 5 µm), mobile phase: A: 0.2% formic acid, B: ACN, C: methanol, gradient elution | The samples of acid and basic hydrolysis were neutralised with NaOH and HCl solutions respectively and diluted 10 times | LOD—0.02 ng mL⁻¹, LQO—0.06 ng mL⁻¹ | [90]       |
| PRULI (ULI)       | Plasma                  | HPLC–MS/MS, column: C18 Diamonsil (200 mm × 4.6 mm, 5 µm), mobile phase: methanol:water:formic acid (70:30:0.2), isocratic elution | Protein precipitation with methanol                                              | LLOQ—0.025 µg mL⁻¹ (ULI)     | [71]       |
| PRULI              | Urine                   | CE with chemiluminescence, capillary: fused-silica capillary (47.5 cm × 75 μm i.d.), buffer: 20 mM sodium citrate, 4 mM citric acid, 10 mM sodium sulfite at pH 6.1 | Dilution with water                                                              | LOD—0.084 µg mL⁻¹            | [91]       |
| GEMI               | Dried blood spots       | HPLC–FLD, column: ZIC-HILIC (100 mm × 4.6 mm, 5 µm), mobile phase: ACN and 10 mM ammonium acetate pH 3.5 (80:20, v/v), isocratic elution | Bried blood spots were extracted with methanol                                     | LLOQ—10 ng mL⁻¹, LLOQ—25 ng mL⁻¹ | [92]       |
| GEMI               | Plasma                  | HPLC–FLD, column: C18 LC-18 symmetry column (150 mm × 3.9 mm, 5 µm), mobile phase: 0.1% TFA: ACN (80:20, v/v), isocratic elution | Ultrafiltration                                                                   | LLOQ—10 ng mL⁻¹, LLOQ—25 ng mL⁻¹ | [72]       |
| GEMI               | Plasma                  | HPLC–UV column: C18 Eurosphere-100 (250 mm × 4.6 mm, 5 µm), mobile phase: methanol: 1% sodium acetate: orthophosphoric acid (65:35:0.5 v/v/v) pH 2.1, isocratic elution | LLE with chloroform and acetic acid mixture (5.4:0.1, v/v)                       | LLOQ—0.3 µg mL⁻¹             | [93]       |
| GEMI               | Plasma                  | HPTLC—10 cm × 20 cm plates 60F₂₅₄ pre-coated with silica gel, mobile phase: ethyl acetate:methanol:ammonia (8.0:4.0:3.0, v/v/v), detection wavelength 254 nm | LLE with chloroform and acetic acid mixture (5.9:0.1, v/v)                       | LLOQ—0.5 µg mL⁻¹             | [93]       |
| GEMI               | Reaction mixture        | HPLC—PDA column: C18 Intersil-ODS-3 V (250 mm × 4.6 mm, 5 µm), mobile phase: 0.1% TFA (pH 2.5): methanol, gradient elution, temperature 27 °C | The reaction mixture was neutralized and diluted                                  | LOD—0.1 µg mL⁻¹, LQO—1.0 µg mL⁻¹ | [94]       |
| GATI               | Urine, blood            | CE with electroluminescence, capillary: fused silica capillary (40 cm × 50 µm i.d.) 12 kV), running buffer: 10 mM PBS pH 5.0 | SPE                                                                              | LOD—0.2 ng mL⁻¹, LQO—0.5 ng mL⁻¹ | [73]       |
Table 1 continued

| Analysed compound | Sample                  | Method                                                                 | Sample preparation                        | LOD/LOQ                  | References |
|-------------------|-------------------------|------------------------------------------------------------------------|-------------------------------------------|--------------------------|------------|
| GATI              | Food samples (muscle, liver and kidney) | HPLC–UV, column: C18 Zorbax SB-Aq (250 mm × 4.6 mm, 5 µm), mobile phase: A: methanol, B: ACN; C: 0.02 M citric acid and 0.03 M ammonium acetate, temperature 35 °C, gradient elution | Accelerated solvent extraction (ASE) with ACN | LOD—3 µg kg⁻¹, LOQ—10 µg kg⁻¹ | [96]       |
| GATI              | Food samples (muscle, liver and kidney) | HPLC MS/MS, column: Hypersil Golden (150 mm × 2.1 mm, 3.5 µm), mobile phase: A: methanol, B: ACN, C: 5 mM ammonium acetate and 0.2% formic acid, gradient elution | Accelerated solvent extraction (ASE) with ACN | LOD—0.3 µg kg⁻¹, LOQ—1.0 µg kg⁻¹ | [96]       |
| GATI              | Plasma                  | HPLC–UV, column: C18 Xterra MS (50 mm × 3.0 mm, 5 µm), mobile phase 0.025 M Na₂HPO₄ (pH 3.0): ACN (80:20 v/v), isocratic elution | Ultrafiltration                           | LOQ—0.1 µg mL⁻¹          | [97]       |
| GATI              | Pharmaceutical formulations | HPLC–UV, column: C-18-DB SUPELCO 516 (250 mm × 4.6 mm, 5 µm), Na₂HPO₄ (pH 3.3): ACN (75:25 v/v), isocratic elution | Extraction from pharmaceutical formulation | LOD—0.507 µg mL⁻¹, LOQ—1.538 µg mL⁻¹ | [98]       |
| GATI              | Tablets                 | HPLC–UV, column: C18 HiQ Sil (250 mm × 4.6 mm, 5 µm), mobile phase 0.01 M KH₂PO₄ (pH 3.0): ACN (70:30 v/v), isocratic elution | Tablets were powdered and diluted with mobile phase | LOD—0.3 µg mL⁻¹, LOQ—0.5 µg mL⁻¹ | [99]       |
| GATI              | Plasma                  | HPLC-FLD, column: C18 Shim-Pack CLC-ODS (250 mm × 4.6 mm, 5 µm), mobile phase: 2.5 mM phosphoric acid: methanol: ACN: TEA pH 2.8 (64:8:15:20:0.2), isocratic elution, temperature 28 °C | SPE                                      | LLOQ—20 ng mL⁻¹           | [95]       |

**Notes:**
- **ACN** acetonitrile
- **ALOD** absolute LOD
- **ASE** accelerated solvent extraction
- **BALO** balofloxacin
- **ALOQ** absolute LOQ
- **BAL** bronchoalveolar lavage
- **BCE** background electrolyte
- **BCG** bromocresol green
- **BPB** bromophenol blue
- **CE** capillary electrophoresis
- **CMPA** chiral mobile phase additive
- **DAD** diode array detector
- **FLD** fluorescent detector
- **GATI** gatifloxacin
- **GEMI** gemifloxacin
- **HPLC** high performance liquid chromatography
- **HPTLC** high performance thin layer chromatography
- **HILIC** hydrophilic interaction liquid chromatography
- **LEVO** levofloxacin
- **LLE** liquid–liquid extraction
- **MBTH** 3-methyl-2-benzothiazolinone hydrazone hydrochloride
- **MEPS** microextraction by packed sorbent
- **MOXI** moxifloxacin
- **MS** mass spectrometry
- **NMR** nuclear magnetic resonance
- **Pazu** pazufloxacin
- **PDA** photodiode array
- **PRU** prulifloxacin
- **SITI** sitafloxacin
- **SPAR** sparfloxacin
- **TBA** tetrabutylammonium acetate
- **TBA·Cl** tetrabutylammonium chloride
- **TBAmBR** tetrabutylammonium bromide
- **TEA** triethylamine
- **TFA** trifluoroacetic acid
- **TROVA** trovafloxacin
- **ULI** ulifloxacin
- **UPLC** ultra performance liquid chromatography
- **UV** ultraviolet
both aqueous [38, 39] and in nonaqueous [40] conditions. The optimum pH of the aqueous solution is about 8.0. The change in pH may influence the response of the detector and it may cause the interaction with capillary wall for pH lower than 2.5. In comparison with chromatographic methods the CE separation is more complicated because there are more factors that influence the resolution of the analysis (pH, voltage, temperature, length of the capillary). The impurities in the sample may absorb in the wall of capillary, thus prolonging the time of the analysis.

The other technique that is applied in LEVO analysis is UV–Vis spectroscopy. This method is suitable for analysis of pure substances and pharmaceutical formulation. LEVO might be detected as the complex with bromophenol blue (BPB) or bromocresol green (BCG) [41] or as itself [42]. Spectroscopy be applied to analyse marketed formulations, as well as for human urine or serum. In this case a fluorescence detector is applied, and the fluorescence is enhanced by SDS micelle [43]. In addition to the UV–Vis spectroscopy, also 1H NMR, adsorptive square-wave anodic stripping voltammetry and synchronous scanning room temperature phosphorimetry may be applied [44–46]. These techniques are rarely used, and they are suitable for analysis of pharmaceutical formulation.

All aforementioned techniques require proper sample preconditioning. The following matrices may be used: serum [26, 43], plasma [20, 21, 23, 24, 27, 28, 31, 32, 34, 45], blood [30], urine [23, 38, 43–45], sputum [36], tissues [33–35], bone [21], bronchoalveolar lavage (BAL) [21], bile [30], water [40], dialysate [28], reaction mixture [19], and pharmaceutical formulation [18, 22, 25, 39, 41–43, 45, 46]. For protein precipitation, an extraction step, dilution is typically applied prior to analysis. Protein precipitation is mainly performed using solvents such as ACN [27, 31, 38], methanol [34], a mixture of ACN and methanol [24], perchloric acid and methanol [26], and trifluoroacetic acid (TFA) [28]. Watabe et al. reported the mixture of methanol and 6% perchloric acid as a precipitating agent. The use of ACN or ethanol resulted in broad or very small peaks; using methanol the supernatant was not clear enough. The use of pure 6% perchloric acid resulted in low recovery. A mixture of ACN results in not complete protein precipitation. The abovementioned proportion resulted in optimum recovery and removal of the protein.

The extraction or precipitation techniques are applied mainly in biological matrices such as plasma, serum, tissue homogenate, BAL, and urine. The dilution can be found often in sample preconditioning of pharmaceutical formulations. The analysis of the levels of LEVO with separation techniques requires also the use of the internal standard. The addition of the internal standard provide the repeatability of the results and improves the precision of the assay.

The limit of detection (LOD) and limit of quantification (LOQ) strongly depended on both the used matrix and applied detector. The LOD and LOQ for pharmaceutical formulations were even of the order of 10−9 g mL−1 for fluorescent detection. The detection limit for the biological matrices such as plasma, urine, and serum were higher. The MS detector was more sensitive for the analyte than UV or FLD; however, for routine clinical practice it is not always necessary to detect very low concentrations because peak and trough concentrations are on the order of mg L−1 [47].

Balofloxacin

To determine BALO, the HPLC technique with MS and UV detection was used [15, 48]. The separation was performed on a C18 column. The organic eluents were ACN and methanol. The inorganic contents were aqueous solutions of ammonium acetate [15] and potassium dihydrogen phosphate [48]. The pH of the mobile phase was slightly acidic [15, 48] (Table 1). The selection of dihydrogen phosphate adjusted to pH 6.5 resulted in good resolution of the analysis and reduction of the tailing [48]. On the other hand Bian et al. [15] tested 10 mM ammonium acetate in different pH conditions (6.65 vs 3.0). The application of the solution with lower pH resulted in better resolution of the analysis and reduction of the tailing of the peak. These differences might be caused by the use of the different organic solvents—ACN and methanol in [48] and [15],
respectively. The sample preparation involved LLE for plasma [15] and dilution for pharmaceutical formulation [48]. In LLE a mixture of dichloromethane-ethyl acetate was used. In comparison with the mixture n-hexane-isopropanol it showed high efficiency and less interference. During the extraction procedure, it was not advisable to use an acid (1 M HCl) or a base (1 M NaOH) because this resulted in higher interference [15]. The LOD and LOQ were lower for MS detection [15]. The quasimolecular ion [M + H]^+ of m/z 390 of BALO was selected for monitoring [15].

**Pazufloxacin**

The methods for determination of PAZU comprise both HPLC [26, 27, 49] and CE technique [50–52]. The analysed matrices were pharmaceutical formulation (tablets), milk [50], and biological fluids, i.e., serum, plasma, urine, muscle homogenate, saliva, gingiva, and crevicular fluid [26, 27, 49, 51, 52]. The applied columns in HPLC were C18 [27, 49] and C8 [26]. The content of ACN in the mobile phase did not exceed 15.5% [26, 49]. The other contents were 0.5% phosphoric acid containing 1% of TEA [49] or 1% TEA solution [26] or 0.1% formic acid at pH 3.0 adjusted with TEA [27]. CE separation was performed at room temperature, and the background electrolyte (BGE) was an aqueous solution of TRIS and phosphoric acid [50, 51]. The other additives were phosphates and β-cyclodextrines [52]. The pH was within the range 5.04–9.00. The length of capillary depended on applied voltage. A lower voltage was applied and a shorter capillary was used (Table 1). The sample preparation involved protein precipitation with methanol [49], ACN [27] or 6% perchloric acid and methanol [26], LLE with dichloromethane [50, 51], and dilution [52]. The LOD depended on a sample preparation—for extraction it was within the range 0.01–0.3 μg mL⁻¹ [49–51], for protein precipitation 0.01–0.1 μg mL⁻¹ [26, 27], and for diluted urine it was the highest at 7 μg mL⁻¹ [52] (Table 1).

**Sparfloxacin**

The method for SPAR analysis comprises spectroscopic and chromatographic techniques. The separation techniques comprise HPLC [35, 53–55], ultra performance liquid chromatography (UPLC) [17], and CE [56]. Gupta et al. [17] compared HPLC with UPLC. It was found that the tailing effect is reduced and the elution time of SPAR was 10-fold lower in UPLC analysis. The number of theoretical plates was three times greater for UPLC than for HPLC The detection applied in chromatographic analysis were UV [17, 53, 54], diode array detector (DAD) [57], and MS [35, 55]. The MRM employed in MS/MS analysis were: m/z 393.2 → 349.3 [35] and m/z 392.9 → 348.7 [55]. The separation was performed on a C18 column. The analysed matrices via separation techniques were plasma, urine, serum, and tissues (muscles) [35, 53–57]. The organic solvent applied in analysis was ACN [17, 35, 53–55, 57]. In isocratic elution the content of ACN was up to 80% [55]; however, in other analyses the content was within the range 12–20% [53, 54]. In addition to ACN, methanol was also used [53]. The other additives in the mobile phase were 0.1% orthophosphoric acid [17], 5% acetic acid [53], NaH₂PO₄ [54], 54 mM formic acid [35], 10 mM CH₃COONH₄ [35, 55], and 0.1% TFA [57]. The addition of acid resulted in slight acidic pH of the mobile phase. The ionic pair reagent was found in [53] and the content was 1% in a mobile phase. In three methods the higher temperature was reported: 30 °C [35], 35 °C [54], and 50 °C [53].

CE was performed in tetraborate buffer with an addition of silica nanoparticles. The pH was about 9.0 [56].

The sample pre-conditioning involved protein precipitation with 20% HClO₄ [53] and ACN [55, 57], LLE with ethyl acetate [54], extraction with ACN/water mixture with addition of phosphoric acid and hexane in muscle tissue [35], and dilution [17, 53, 56]. The addition of a small amount of phosphoric acid increased the recovery of SPAR from the muscle tissue. The authors also used the formic acid; however, the recovery in this case was lower [35]. The detection limits strongly depended on the applied detection. The lowest were noted for MS detection [55] (Table 1).

**Fourth Generation Fluoroquinolones**

The representatives of fourth generation fluoroquinolones are MOXI, TROVA, sitafloxacin (SITA), prulifloxacin (PRULI), gemifloxacin (GEMI), GATI [12]. MOXI is characterized by a wide range of activity. The activity comprises Gram-negative and Gram-positive bacteria, such as *Staphylococcus*, *Streptococcus*, *Enterococcus*, and also atypical bacteria and anaerobes [58–61]. It is used in the treatment of conjunctivitis, keratitis, pre- and postoperatively to control infections of the eyes. LEVO is used in CAP and in multidrug resistant tuberculosis (MDR-TB) treatment [62]. The killing effect on slow replicating bacilli in the tissues is an important factor that shortens MDR-TB treatment and, therefore, MOXI is often added to standard therapy [63–66]. TROVA exhibits a broad activity spectrum against Gram-positive and Gram-negative bacteria. It is used mainly in veterinary medicine—it was withdrawn from the market in 1999 due to incidents of idiosyncratic hepatotoxicity [67, 68]. SITA is active against Gram-positive and Gram-negative bacteria, *Chlamydia* spp., and *Mycoplasma* spp. It also shows activity against quinolone-resistant methicillin-resistant *S. aureus*, *Pneumococcus* spp.
and *Pseudomonas* spp. [69]. PRULI is a prodrug of ulifloxacin (ULI). PRULI is rapidly metabolized by paraoxogenases to ULI. It is applied in simple cystitis treatment, acute exacerbation of chronic bronchitis, and lower urinary tract infections in children and adults [70, 71]. GEMI is a broad bactericidal spectrum drug. It has particularly enhanced activity against Gram-positive organisms. It also shows fourfold higher bactericidal activity against *S. pneumoniae* than MOXI and is active against *H. influenzae* and *M. catarrhalis* and the atypical organisms *L. pneumophila, Chlamydia* spp., *Mycoplasma* spp. It is also applied in urinary tract infections [72]. GATI is active against Gram-positive and Gram-negative bacteria. It is active in vitro against clinically important pathogens such as penicillin-resistant *S. pneumoniae* [73].

**Moxifloxacin**

Moxifloxacin may be analysed by many analytical techniques. The predominant ones are reversed phase HPLC with different detectors such as UV [20, 24, 54, 60, 74, 75], FLD [27, 29, 76–79], MS [80–82], and DAD [57, 66]. There are also reported methods for CE [32, 83, 84]. A simple method involving UV–Vis spectroscopy is applied in analysis of pharmaceutical formulation and pure substance [85].

The main constituent of the mobile phase in HPLC separation was the phosphate buffer [24, 29, 54, 66, 74–76, 78]. The concentration of the phosphate buffer was within the range 10–50 mM; however, there is also a reported method with a high concentration of sodium phosphate (0.25 M) [78]. The other constituent of the aqueous phase might be carboxylic acid such as citric acid [20], formic acid [27], acetic acid [81], TFA or its anhydride [81]; organic salts such as ammonium acetate [81] and SDS [20]. Chan et al. [77] reported the use of TFA because it doesn’t affect the fluorescence signals. In many methods an ion pair reagent was the constituent of the mobile phase. The most common used was TEA [60, 66, 74, 75, 80]. The concentration was in the range 0.03–2.00%. The lowest concentration was applied in the method with MS-detection [80]. For the other detectors the minimum concentration of TEA was 0.1%. The other ion pair reagents were TBAAC [20], TBAmBr [29], and tetrabutylammonium chloride (TBA-Cl) [77, 79]. The ion pair reagent reduces the tailing of the peaks due to the interaction with the silanol groups. It reduces both the availability of free stationary phase silanols and the analyte’s interaction with them. The addition of ion pair reagent should be as low as possible. The high content causes a long column equilibrating time, and it is difficult to wash off the column. The high tailing is also observed for pH 4.5 and 5.5. The negatively charged silanol groups from the stationary phase and positively charged amine group of MOXI are responsible for it. The decrease of pH value to 3.5 results in reducing of peaks tailing. The silanol groups above pH 3.5 are ionized and interact with 1° and 2° amines [74]. The content of the water-based phase in isocratic elution was 57–95%. The organic solvents applied in the chromatographic analysis were ACN [20, 24, 29, 54, 57, 60, 76, 78, 79, 81, 82], methanol [66, 74, 80], or both [27, 75, 77]. The pH of the mobile phase was acidic 2.5–6.0. Laban-Djurdević et al. [78] optimized the condition of the analysis by the response surface method in two factor space. The statistical analysis was performed by the Statistica v.6 software and it occurred that the most important factors influencing retention time and resolution were ACN content and pH of the mobile phase. Less significant occurred to be the ionic strength of the phosphate buffer. It was observed that response surface possess a relatively flat maximum situated between 10 and 15% ACN and pH within the range 3.0–4.5.

In MS/MS analysis the following MRM transitions were applied: *m/z* 402.1 → 260.0 [80], *m/z* 402.0 → 358.2 [81], and *m/z* 402 → 384, *m/z* 402 → 358 [82].

The next separation technique applied in MOXI analysis is CE. The BGE in reported method consisted of buffers (both organic and inorganic), salts, and TEA [39, 83, 84]. In order to improve the resolution for enantiomers the sulfated γ-cyclodextrin were applied [84]. The pH depended of the constituents of the mobile phase and it was both acidic or base. The measurements were performed at the room temperature.

Ashour et al. reported the simple technique for analysis of MOXI in both pharmaceutical formulation and pure substance [85]. The method was based on the coupling reaction of MOXI with 3-methyl-2-benzothiazolinone hydrzone hydrochloride monohydrate (MBTH) in the presence of Ce(IV) ions. This method is suitable for kinetic measurements. Djurdevic et al. [60] and Cruz et al. [84] reported the methods that were also suitable for impurities analysis. They are based on HPLC and CE. The HPLC method was suitable for analysis of impurities and forced degradation products [60]. The CE method was suitable for determination of S,S-, R,R-, R,S-, and S,R-diastereoisomers of MOXI. The active one was S,S-isomer, the other ones were potential chiral degradation products of MOXI [84].

The preconditioning of the sample involves the following techniques such as extraction [29, 54, 75, 79, 81–83], protein precipitation [24, 27, 57, 76, 80], dilution [39, 60, 66, 74, 77, 79, 84, 85], and filtration [20, 78]. The extraction techniques comprise classical LLE with dichloromethane [75, 83], ethyl acetate [54], SPE [79, 82], and others such as online extraction on pre-column [29] and extraction with cyanoimipramine [81]. The protein precipitation was performed by ACN [27, 57, 77], methanol [80], mixture of ACN and methanol [24], and HClO₄ [76].

The analysed matrices were plasma [20, 24, 27, 54, 57, 75, 78, 80, 82], serum [29], blood [81], saliva [76], muscle

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[83], vitreous and aqueous humor [77, 79], and pharmaceutical formulations (eye drops, tablets), as well as in pure substance [39, 60, 66, 74, 84, 85]. The detection of MOXI in biological matrices involved mainly extraction or protein precipitation step. However, ultrafiltration with SDS as an displacing agent or microfiltration was also applied. The displacing reagent was used as a drug releasing factor from the proteins. It enhances the protein solubility and minimizes binding with the drug. The different concentrations of SDS were tested, and it was found that the most optimum was 10 mM SDS and phosphoric buffer adjusted to pH 3.0. Addition of SDS also increased the fluorescence intensity [78]. The preconditioning of the sample in pharmaceutical formulation was dilution. Internal standards were used in the most of the methods.

The LOD and LOQ mainly were of the order of μg mL⁻¹. However, both MS and FLD or UV detection enabled to detect the levels of ng mL⁻¹ (Table 1).

Trofavacin

TROVA is analysed by HPLC with both UV and FLD [20, 27, 67, 86]. The separation is performed on a C18 column. The organic solvent applied in the analysis was ACN [20, 86] or as a mixture with methanol [27, 67]. Ion-pair reagent used were TBA as hydroxide [67], hydrogensulfate [86], or acetate [20] and TEA [27]. The inorganic content was dihydrogen-phosphate sodium [67], sodium phosphate [86], or 0.1% formic acid [27]. The other substances found in the mobile phase were SDS and citric acid [20]. The pH of the mobile phase was slightly acidic. The analysed matrices were plasma and urine. The sample preconditioning was protein precipitation (for plasma or serum) with ACN [27], a mixture of ACN and perchloric acid [86], and 20% perchloric acid [67]. The other technique was ultrafiltration with 0.5% SDS solution preconditioning [20]. Urine was diluted prior to analysis [86]. The LODs were similar for the mentioned chromatographic methods with protein precipitation. The detection limit was higher for urine. TROVA was also analysed by differential-pulse adsorptive stripping voltammetry [68]. In this case the sample preconditioning was filtration and LOD was higher than for the formerly reported methods (Table 1).

Sitafloxacin

SITA was analysed by chromatographic methods with the mass detection [69, 87]. The MS/MS analysis was based on the following MRM transitions: m/z 410.1 → 392.1 [87] and m/z 410.1 → 392.2 [69]. The composition of the applied mobile phase in reported methods was similar—it was a mixture of methanol and 0.1% formic acid. In the method where the content of formic acid solution was higher (62 vs. 54%) the temperature of the separation was also higher, i.e. 40 vs. 35 °C. In both cases the protein precipitation was the method for sample preconditioning. The precipitating agent was methanol with a 0.1% addition of formic acid [87] or isopropanol [69]. The quantitation limit was lower in case of [87] (Table 1).

Pruflifloxacin

PRULI is a produg of ULI. Some reported methods for PRULI determination also reports the determination of ULI. ULI was also considered as an impurity of PRULI [88]. Both compounds were analysed by HPLC techniques with UV [70, 88, 89] or MS detection [71, 90] and CE [91]. The following MRM transitions were observed for PRULI in MS/MS analysis: m/z 462 → 444; m/z 462 → 418; m/z 460 → 360; m/z 462 → 350 [90]. These transitions are helpful in analysis of degradation products of PRULI. For ULI the following MRM in MS/MS detection was employed m/z 350 → 248 [71]. The analysed matrices were tablets [70, 88], degradation products [90], aqueous human humor [89], plasma [71], and urine [91]. The separation was performed on C18, C8, and HILIC columns. The mobile phase consisted of ACN [70, 88–90] and methanol [71] as an organic content. ACN can be replaced with alcohol; however, in this case its content must be higher to achieve the same degree of retention on HILIC column [88]. The other contents of mobile phase were dihydrogen potassium phosphate [70], ammonium acetate [88], phosphoric acid [89], formic acid in MS detection [71, 90]. The BGE in CE consisted of sodium citrate, citric acid, and sodium sulfite [91]. The sample preconditioning involved pulverization and dissolution with a proper solvent in case of tablets [70, 88], dilution in case of aqueous matrices [89–91], protein precipitation with methanol [71]. The limits of detection depended on the applied matrix and the detector. The lowest were for the MS detection for PRULI [90]. The same type of detection for ULI resulted in higher LOD [71]. In the methods where PRULI and ULI were detected simultaneously the detection limits were lower for PRULI [88] (Table 1).

Gemifloxacin

GEMI was analysed by HPLC and high performance thin layer chromatography (HPTLC). The HPLC methods involved FLD, UV, and PDA detection [72, 92–94]. The HPTLC plates were detected in UV light [93]. The applied columns were C18 [72, 93, 94] and HILIC [92]. As in the previously reported method the applied organic solvents were ACN [72, 92], methanol [93, 94]. The aqueous solutions contained 10 mM ammonium acetate [92], 0.1% TFA [72], sodium acetate [93], orthophosphoric acid [93]. Ethyl acetate and ammonia were used in HPTLC separation [93].

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The analysed matrices were dried blood [92], plasma [72, 93], and reaction mixture [94]. The sample preconditioning involved extraction with methanol for blood spots [92], ultrafiltration [72], LLE [93], and dilution [94]. The quantification limits were lower for FLD detection [72, 92] than for UV and PDA detection [93, 94] (Table 1).

**Gatifloxacin**

In GATI analysis were applied HPLC and CE. The HPLC techniques involved FLD [20, 27, 29, 79, 95], UV detection [20, 24, 25, 54, 96–99], and MS detection [96]. The detection in CE involved UV detection [39, 52, 56], capacitively coupled contactless conductivity detection [83] and electroluminescence [73].

The chromatographic analysis was performed on C18 column. The organic eluent was ACN [20, 24, 25, 27, 29, 54, 79, 95–97], methanol [27, 95, 96]. Ion pair reagents were TBAA [20], TEA [25, 27, 95], TBAmBr [29], TBA-Cl [79]. The other constituents were phosphates [24, 29, 54, 97–99], SDS [20], citric acid [20, 96], 0.1% FA [27, 96], TFA [79], ammonium acetate [96], phosphoric acid [95]. The pH of the mobile phase was acidic. The elution was both isocratic [20, 24, 29, 54, 79, 95–97] and gradient [27, 96]. HPLC analysis was performed at ambient temperature with the exception of [54, 96] and [95], where it was performed at 35 and 28 °C, respectively. CE separation was performed on PBS [73], TRIS/hydrochloride [39], tetraborate buffer [39], phosphate buffer with cyclodextrins [52], disodium tetraborate with silica nanoparticles [56], tartaric acid, and sodium acetate [83]. The following MRM transitions were employed in MS/MS analysis: mlz 375.9 → 320.0 and mlz 375.9 → 260.9 [96].

The analysed matrices were tablets [39], pharmaceutical formulations [25, 98], serum [29, 56], plasma [20, 24, 27, 53, 54, 95, 97], blood [73], urine [52, 53, 73], muscle [83], aqueous and vitreous humor [79], and food samples [96].

The sample preconditioning involved pulverization and dissolution [25, 39, 99], ultrafiltration [20, 97], online extraction on column [29], protein precipitation with ACN [27] or methanol or a mixture of ACN and methanol [24], dilution with a proper solvent [52, 56, 79], LLE [54, 83, 98], SPE [73, 79, 95], and ASE (accelerated solvent extraction) [96]. Fu et al. [73] reported that SPE lead to successful clean-up of the sample and that no unacceptable interference was observed during the analysis. Tasso et al. [95] reported the method of online SPE combined with HPLC. The biological sample was injected onto a cartridge and it was eluted cleaned up using proper solvents and washed off on a column. The detection and quantification limits depended on the applied detection and there were lower for MS detection (Table 1).

**Conclusion**

This paper presents information about methods involving different analytical and separation techniques and, therefore, might be helpful in the selection of procedures for the levels determination of the antimicrobial agent.

The fluoroquinolones posses two groups that can interact with protons—amine and the carboxyl groups. They are strongly ionized compounds due to their zwitterionic nature. It may cause that analysis of these substances with separation techniques becomes complicated. The tailing of peaks and also poor resolution on the column are the main problems that can be encountered during analysis. The most commonly used organic solvents are ACN and methanol in gradient or isocratic elution. ACN has more elution strength than methanol and often causes peaks to appear on the chromatogram. The addition of methanol may influence the resolution between peaks. The separation of fluoroquinolones is often performed on reversed phase. The applied columns are mainly C18, C8, and C4. The other columns that may be used in analysis are HILIC. They are the alternative to RP-HPLC for separation of hydrophilic ionized solutes. In this case the content of at least 80% of organic solvent (mainly ACN) is required. This type of column is desirable for separation in MS analysis. The organic solvent evaporates easily and results in low content of aqueous solution, which is not as volatile as ACN or methanol. One of the constituents of the mobile phase applied in the analysis of the fluoroquinolones is the solution of ion-pair reagents (TEA, TBAA, SDS, or others). The content of this type of reagent results in a longer column equilibrating time and may lead to problems with column maintenance. On the other hand, ion-pair reagents cause better interaction of the analyte with the stationary phase. When considering the composition of the mobile phase, researchers should take into consideration these two facts. The ion-pair reagent should be added to the mobile phase when the addition of the buffer at the proper pH value does not suppress the peak tailing effect or does not provide a good resolution of the analytes. The applied column, the addition of other contents such as organic or inorganic salts, ion-pair reagent, and also the proper value of pH influence the shape of the peak and may have an impact on LOD and LOQ. The other things to consider are the sample preconditioning and the applied detector. In quantitative analysis the applied method must be appropriate for the predicted level of the analyte, used matrix, and the aim of the analysis. The analysis performed in biological fluid will be characterized by higher LOD or LOQ than those performed in pharmaceutical formulation or aqueous solution, and they require proper
preconditioning prior to analysis. The LOD and LOQ is higher in the methods involving protein precipitation in the preconditioning step than in the methods in which the extraction step (LLE or SPE) is involved. The extraction techniques are more laborious in sample preparation, but they are useful in the detection of the lower concentration of the analyte in a sample. In the analysis of pharmaceutical formulation there is no urgent need to apply the aforementioned preconditioning steps—the dilution is sufficient. The analysis in this case may be performed by UV, Vis, and fluorescence spectroscopy. Deproteinization step by protein precipitation is the least complex procedure for sample preconditioning when the removal of the proteins is required. LLE or SPE are more complex techniques because they involve the evaporation of the solvent and dissolution of the sample. These steps are the points where possible mistakes can be made. Also, they are time consuming and more expensive because they involve the use of additional reagents and equipment. The sample preparation should be as simple as possible and fitted to the matrix. There are matrices that require more complex sample precondition like tissue homogenates. In this case not only proteins should be removed, but also lipids. Protein precipitation should be done with a solvent that prevents too much solidification which may lead to the decrease of the recovery. The analyte might be trapped on the precipitate. In this case the addition of water based solution can be applied. The other advantage of protein precipitation is that the recovery of the analyte is greater than for the extraction techniques (SPE and LLE). Moreover, the difference of the hydrophobicity of the analytes must be also taken into consideration during extraction procedures. The separation techniques must be applied when there is more than one analyte that can be detected under the analysis conditions. The methods that apply fluorescent detection or mass detection are characterized by lower LOD and LOQ. The combination of mass detection with extraction step results in an LOD on the order of pg mL\(^{-1}\). MS analysis requires an organic acid as a protonating agent. The most common are formic acid, acetic acid alone or in a mixture with ammonium formate or acetate. The applied MS detection was a tandem MS/MS in most cases. The analysis of the level of the antibacterial drugs is essential from the clinical point of view in order to avoid the resistance of the microorganism on therapy. In the clinical analysis, where the fast result is required to improve the treatment with antimicrobial agent, the protein precipitation combined with HPLC should be applied. However, if it is not possible to apply MS detection, FLD or UV detection with the addition of an internal standard is a suitable technique for fast and inexpensive analysis. Fluoroquinolones of the third and fourth generation are the antibacterial agents for which the concentration in blood and other fluids are on the order of mg L\(^{-1}\) and the HPLC techniques with FLD or UV detection are suitable. The use of an internal standard also compensates for the loss of analyte during the extraction step and provides repeatable results.

Compliance with Ethical Standards

Conflict of interest There are no conflict of interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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