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REVIEW

Analytical methods for the determination of certain antibiotics used in critically ill patients

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Abstract: In this literature review, we will present the latest reported methods that have been developed for determination of certain antibiotics which are used for the treatment of serious infections that may affect critically ill patients such as cefepime, levofloxacin, vancomycin and gentamicin in their pure form, pharmaceutical dosage form, and in biological samples.

Keywords: cefepime, levofloxacin, vancomycin, gentamicin, antibiotics

1 Introduction

There is a global trend towards individualized antibiotic dosing for critically ill patients. Since critically ill patients are subjected to different pathophysiological changes that may alter the pharmacokinetics of the antibiotics which does not account for these changes[1,2]. Any change in the pharmacokinetics of these antibiotics may lead to sub therapeutic doses or over doses which would end with poor clinical outcomes[3,4]. Therefore using the traditional antibiotic dosing would lead to failure of the therapy and increase the resistance to these antibiotics because of the inability to achieve the pharmacokinetic/pharmacodynamics targets associated with the optimum antibiotic efficacy and safety. Applying individualized antibiotic dosing increase the demand to sensitive, accurate and precise methods of analysis of these antibiotics, either alone or in combination.

2 Cefepime

Cefepime[5] as Figure 1 shows.
Molecular formula: $C_{19}H_{24}N_6O_5S_2$
Molecular weight: 480.56 g/mol
Chemical name: (6R,7R)-7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-3-[(1-methylpyrrolidin-1-ium-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

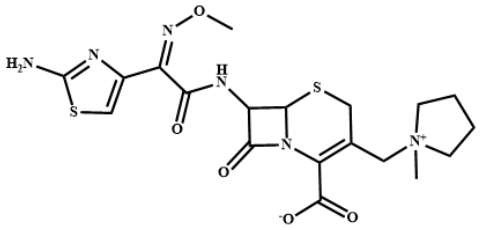

Figure 1. Cefepime

Physical properties: It is an official drug in USP[6] and BP. A white or almost white, non-hygroscopic, crystalline powder. Freely soluble in water and in methanol, practically insoluble in methylene chloride. Store in airtight containers. Protect from light[5].

Melting point: 150°C

Mechanism of action: Cefepime is a broad-spectrum fourth-generation cephalosporin with greater activity against both gram-negative and gram-positive organisms including pseudomonas aeruginosa. It is bactericidal and, like the penicillins, they act by inhibiting synthesis of the bacterial cell wall[7].

Uses and administration: It is used in the treatment of infections of the urinary tract, respiratory tract, and skin[7].

2.1 Methods of determination

2.1.1 Pharmacopeal method

BP: Gradiant chromatographic method using a mobile phase consists of acetonitrile and a 0.68 g/L solution of potassium dihydrogen phosphate previously adjusted

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to pH 5 with 0.5 M potassium hydroxide and column with end-capped octadecylsilyl silica gel (0.25 m, 4.6 mm and 5 μ). The flow rate was 1 ml/min and detection spectrophotometrically at 254 nm[5].

USP: As mentioned above in BP[6].

2.1.2 Chromatographic methods

A novel HPTLC method was developed for the analysis of four β-lactams; cefaclor, cefotaxime, Cefepime, and meropenem, in human plasma using A mixture of ethylacetate: methanol: deionized water: formic acid (60: 30: 15: 1, by volume) as a solvent and the detection was carried out at 270 nm[8].

Analytical separation of Cefepime, meropenem, piperacillin, tazobactam, meropenem, and piperacillin with LC-MS/MS was carried out using C18 100 A LC column (50 mm $\times$ 2.1 mm $\times$ 2.6 μm) coupled with Ultra UPLC Evo C18 cartridge using a mobile phase composed of a mixture of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid) with flow rate at 0.25 ml/min. The total run time was 7.0 min, and gradient elution was performed as follows: 2% solvent B (0 to 0.3 min), 40% solvent B (0.3 to 2.8 min), and a hold at 2% solvent B until the end of the run[9].

Patil *et al*. performed LCMS/MS bioanalytical method for the simultaneous quantification of zidebactam and Cefepime in dog plasma. It was achieved on a Luna HILIC 200A, 100 $\times$ 2.0 mm, 3 μm with a guard column and the mobile phase composed of a mixture of buffer solution (10 mM ammonium formate in water pH adjusted to 3.5 with formic acid) and acetonitrile (28:72 v/v)[10].

Micellar electrokinetic capillary chromatography with UV detection and LC-MS was described for monitoring of Cefepime in urine. MEKC analyses were performed in uncoated fused-silica capillaries of 50 μm id and 60 cm length and the BGE (separation medium) was composed of 6 mM $Na_2B_4O_7$, 10 mM Na_2HPO_4, and 75 mM SDS (pH 9.1). LCMS analyses were performed for serum using a PFP Nucleodur HPLC column together with HP series 1100 instrument coupled to single quadrupole LCMS System with flow rate at 0.5 mL/min using solvent A which consisted of 5 mM ammonium formate (pH 3) and solvent B comprising 100% acetonitrile that was increased from 5 to 20% in the time range of 010 min (linear gradient)[11].

High Performance Thin Layer Chromatographic method for the simultaneous estimation of Cefepime and tazobactam in combined parenteral dosage form was achieved on aluminium plates pre-coated with silica gel 60 F254 as the stationary phase and the mobile phase used was in the combination of chloroform: methanol: water: acetic acid (6:3:1:0.1v/v). Densitometric analysis was carried out at 254 nm[12].

(UFLC) method was developed for simultaneous determination for Cefepime, cefotaxime and ceftriaxone in pharmaceutical formulation using Prodigy, ODS3, 5 μm, (250 $\times$ 4.6 mm) column connected with PDA detector set at 260 nm and a mobile phase consisted of acetonitrile and 0.025M KH_2PO_4 pH 3.0 in the ratio of 15:85 (by volume) at a flow rate of 1.5 ml/min[13].

LC-MS/MS was presented for the simultaneous quantification of Cefepime and tazobactam in dog plasma. The method was achieved on 100 $\times$ 4.6 mm, 3 μm column using a mixture of buffer (25 mM ammonium formate in water pH adjusted to 3.2 with formic acid) and acetonitrile (25:75 v/v) was used as mobile phase at a flow rate 1 ml/min and MS/MS analysis in positive ion mode[14].

RP-HPLC method was described for the quantification of Cefepime and amikacin in Pure and pharmaceutical formulations. This method was carried out on C18 (250 mm $\times$ 4.6 mm, 5 μm) column using a mobile phase consists of methanol: acetonitrile: acetate buffer 75:20:05 (v/v) at pH 5.1 with flow rate 1 ml/min and detection wave length was 212 nm[15].

LCMS/MS assay using protein precipitation for cleanup followed by a gradient separation within 6 min was carried out to simultaneously determine five antibiotics in human plasma; Cefepime, daptomycin, meropenem, piperacillin/tazobactam and Vancomycin[16].

UHPLC method using UV detection was described for the simultaneous quantification of eight β-lactam antibiotics in human plasma, including four penicillins, amoxicillin, cloxacillin, oxacillin, and piperacillin, and four cephalosporins, cefazolin, Cefepime, cefotaxime, and ceftazidime. Proteins were precipitated by acetonitrile containing 0.1% formic acid. Separation was carried out on a pentafluorophenyl column using a mobile phase consisted of phosphoric acid (10 mM) and acetonitrile in gradient elution mode at a flow rate of 500 μl/min. Detection was set at 230 nm for amoxicillin, cloxacillin, oxacillin, and piperacillin and 260 nm for cefazolin, Cefepime, cefotaxime, and ceftazidime within 13 min[17].

Chromatographic separation was achieved on PrincetonSPHER-100 C-18 column 250 mm $\times$ 4.6 mm i.d. 5 μm) for the simultaneous determination of Cefepime Hydrochloride and tazobactam sodium in bulk and pharmaceuticals using a binary mobile phase composed of 25mM potassium dihydrogen phosphate buffer, pH 6.2 and acetonitrile (94: 6, v/v) with flow rate of 1 mL/min and at a wavelength 210 nm[18].

RP-HPLCUV method with solid phase extraction was reported for the quantification of Cefepime in total nutrient admixtures utilizing C18 column with a mobile phase consisting of ammonium acetate, acetonitrile and methanol. The flow rate was at 0.9 mL/min and the wave-

length was 257 nm[19].

Verdier *et al*, developed HPLC-UV for the simultaneous determination of 12 beta-lactam antibiotics (amoxicillin, Cefepime, cefotaxime, ceftazidime, ceftriaxone, cloxacillin, imipenem, meropenem, oxacillin, penicillin G, piperacillin, and ticarcillin) in human plasma. Atlantis T3 analytical column with a linear gradient of acetonitrile and a pH 2 phosphoric acid solution was used for separation after protein precipitation was done by acetonitrile using either at 210 nm, 230 nm, or 298 nm according to the compound[20].

Another HPLC method for the separation of seven cephalosporins [Cefepime, ceftazidime, ceftizaxime, ceftriaxone, cefotaxime, cefixime and cefoperazone] in human plasma and amniotic fluid was carried out with XTerra C18 (250 mm × 4.6 mm, 5 μm i.d.) column, 40 mM phosphate buffer, pH 3.2, 18% MeOH, 0.85 mL min1 flow rate and 32 °C column temperature. Gradient elution with MeOH was applied[21].

HPLC method was developed for the simultaneous determination of Cefepime and cefazolin in human plasma and dialysate using a C18 column with an aqueous mobile phase consisted of dibasic potassium hydrogen phosphate (pH 7.0) and methanol gradient at a flow rate of 1 mL/min[22].

HPLC method was presented for the estimation of Cefepime and ceftazidime in plasma and dialysate-ultrafiltrate samples obtained from intensive care unit patients undergoing continuous veno-venous hemodiafiltration involved protein precipitation with acetonitrile, followed by washing with dichloromethane to remove apolar lipophilic compounds and separation was achieved on a μBondapak C18 (30 cm × 3.9 mm × 10 μm) with UV detection. The mobile phase consisted of acetate buffer: ACN. The flow rate was at 2 ml/min[23].

RP-18 column (250 mm × 4 mm, 5 μm particles) was described for the analysis of Cefepime in several biological matrices utilized a mobile phase, phosphate buffer (pH 7) 10 mMmethanol 75:25, was always freshly prepared at a flow rate 1 mL/min with detection at 256 nm[24].

Another HPLC method utilizing a C18 column and acetonitrile/0.1M phosphoric acid/sodium hydroxide buffer (pH 3.0)/0.01M n-octylamine (pH 3.0) as mobile phase in gradient mode was described for the simultaneous estimation of the cephalosporin Cefepime and the quinolones, garenoxacin, Levofloxacin and moxifloxacin coupled with a diode-array UV detector set at 256 nm for Cefepime, 292 nm for Levofloxacin, 294 nm for moxifloxacin and 282 nm for garenoxacin at a flow rate 1 ml/min[25].

Cefepime was separated on a C18 column (LC18, 15 cm by 4.6 mm) with a diode array detection at 260 nm. The mobile phase (1 ml/min) composed of 0.2 M borate adjusted to pH 6.8 (20 ∘C) with NaOH and methanol in the ratio (92:8, v/v)[26].

B. Calahorra *et al*, performed HPLC method using a reversed-phase encapped column (Hypersil BDS C18) and the samples, after protein precipitation, were eluted with a mobile phase of acetonitrileacetate buffer, pH 4 (2.8:97.2, v/v). The detection wavelength was 254 nm[27].

An isocratic RP-HPLC method was reported for the qautification of Cefepime levels in plasma and vitreous fluid. Seperataion of Cefepime and the internal standard cefadroxil was achieved on a Shandon Hypersil BDS C18 column by using a mobile phase composed of 25 mM sodium dihydrogen phosphate monohydrate (pH 3) and methanol (87:13, v/v) with UV detection at 270 nm[28].

HPLC method for the determination of Cefepime was achieved on a reversed-phase Ultrasphere XL-ODS column (75 × 4.6 mm I.D.) employing 7% acetonitrile in 20 mM ammonium acetate (pH 4) as a mobile phase and detection was by UV absorbance at 254 nm[29].

2.1.3 Spectrophotometric methods

Extractive spectrophotometric method based on the ion-association reaction between Cefepime Hydrochloride and a bromophenol blue, in phosphate buffer pH 3.0 was achieved by forming a reddish orange chromogen extracted in a chloroform layer showing λ max at 417 nm[30].

Another spectrophotometric method was achieved by the reaction of cephalosporin's with ninhydrin reagent in the presence of sodium molybdate by adjusting the pH to 5.5 using citrate buffer at a temperature of 100 ∘C and the resulting ruhemann's purple product having the absorption maximum at 570 nm[31].

Spectrophotometric absorption correction method was performed for simultaneous quantification of Tazobactam and Cefepime in combined tablet dosage form using 0.1 N NaOH as solvent. Absorbance correction based on the property of additivity of absorbances using wavelengths at 259 nm and 306 nm[32].

Another spectrophotometric method was achieved by the formation of red coloured complex with 1, 10 phenonthroline in presence of Ferric nitrate in aqueous medium showing maximum absorption at 515 nm[33].

Elazazy *et al*, developed spectrophotometric method for the determination of micro amounts of Cefepime hydrochloride and cefuroxime sodium based on reduction of 2,3,5-triphenyltetrazolium chloride (TTC) by the cited drugs in slightly alkaline medium leading to formation of a highly colored formazan derivative and Absorbance measurements were made at 483 nm[34].

First derivative Spectrophotometric method for the simultaneous determination of Cefepime Hydrochloride

and Amikacin Sulphate in combined Parentral dosage form was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectra were obtained in 0.1N HCl and the estimations were made at 220.0 nm (ZCP of Cefepime Hydrochloride) for Amikacin Sulphate and 294.0 nm (ZCP of Amikacin Sulphate) for Cefepime Hydrochloride[35].

Another method is based on the reduction of the chromogenic agent, ammonium molybdate (Mo^{6+}) into molybdenum blue (Mo^{5+}) by the examined drugs in sulphuric acid medium and by aid of heating in boiling water bath. The resulting "blue coloured" product showed a maximum absorbance at 695-716 nm[36].

2.1.4 Miscellaneous methods

Spectrofluorometric method was described for determination of some cephalosporins including Cefepime. The amine group in these drugs react with acetylacetone and formaldehyde reagents via a Hantzsch reaction. The fluorescence intensity of the products were measured at an emission wavelength of 482 nm after excitation at a wavelength of 415 nm[37].

Square-wave voltammetric method on a hanging mercury drop electrode was proposed for indirect determination of Cefepime by forming schiff base in its pharmaceutical formulations[38].

A.M.J. Barbosa *et al*, represented solid silver mercury amalgam electrodes (AgSAEs) as a suitable alternative to mercury electrodes due to their similar electrochemical properties and non-toxicity of the amalgam material. In this study the use of silver solid amalgam was reported as working electrode to estimate Cefepime in human milk sample. Square-wave voltammogram registered for Cefepime in brittonrobinson buffer at pH 2.5, presented two well-defined reduction peaks, at -0.28 V (peak 1C) and -0.45 V (peak 2C)[39].

A simple and rapid differential pulse polarographic method was developed for the determination of Cefepime and cefpirome in pharmaceutical formulations and spiked urine samples using the standard addition method[40].

Differential pulse voltammetry (DPV) along with cyclic voltammetry (CV) was performed by the application of microfabricated chip with integrated Pt microelectrodes, for the electrochemical detection (ECD) of Cefepime. In this analysis, electro-oxidation of Cefepime was investigated on an unmodified Pt microelectrode in acetate buffer at pH 4.5[41].

3 Levofloxacin

Levofloxacin[6] as Figure 2 shows.

Molecular formula: $C_{18}H_{20}FN_3O_4$

Molecular weight: 361.4 g/mol

Chemical name: (-)-(S)-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

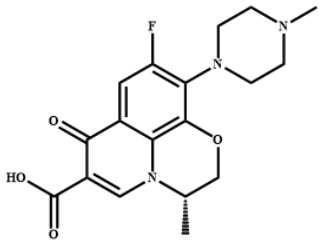

Figure 2. Levofloxacin

Physical properties: It is official drug in USP only. Levofloxacin is a light yellowish - white to yellow - white crystal or crystalline powder. It is soluble in water.

Melting point: 225-227°C

Mechanism of action: It is bactericidal third-generation fluoroquinolone and acts by inhibiting DNA gyrase and topoisomerase IV, which are essential enzymes in the reproduction of bacterial DNA. It has a broader spectrum of activity and is more potent in vitro than the non-fluorinated quinolone with an activity against gram-negative bacteria including pseudomonas aeruginosa and with less activity against gram-positive organisms[7].

Uses and administration: It is used for the treatment of uncomplicated infections of the urinary tract, skin, and bacterial prostatis with usual doses range from 250 to 500 mg once or twice daily for 7 to 14 days depending on the severity and nature of the infection. A dose of 750 mg once daily for 7 to 14 days may be used for hospital-acquired pneumonia[7].

3.1 Methods of determination

3.1.1 Pharmacopeal method

USP: Chromatographic method carried out on a 4.6 mm × 25 cm; 5 μm packing L1 mobile phase consists of methanol and a buffer composed of ammonium acetate, cupric sulfate, pentahydrate and L-isoleucine in water at a flow rate 0.8 ml/min[6].

3.1.2 Chromatographic methods

Levofloxacin degradation products were identified in this study using HPLC-MS and TOF detectors while the quantitative assay was done by a validated HPLC method using isocratic elution with a mobile phase composed of acetonitrile and 0.4% triethylamine solution adjusted to pH 2.5 with orthophosphoric acid (24:76, v/v) at a flow rate 1 ml/min on a column (250 × 4 mm, 5 μm) with guard column (4 × 4 mm, 5 μm) and with UV detection at 295 nm[42].

Andrzej Czyrski performed HPLC method for the determination of levofloxacin on a column (125 × 4 mm, 5 μm) with guard column (4 × 4 mm, 5 μm) using a mobile phase consisted of acetonitrile and 0.4% aqueous solution

of triethylamine (pH 3.0) (by the addition of concentrated phosphorous acid) in the ratio (24:76 v/v) at a flow rate was 1 mL/min, and the detection at λ=295 nm[43].

Ultra-fast HPLC-DAD for Levofloxacin quantification in rabbit aqueous humor was developed and applied to a pharmacokinetic study, Separations were obtained on a XB-C18 column (100 mathringA, 100 mm × 4.60 mm, 2.6 μm) with an isocratic mobile phase consisting of 18% acetonitrile and 82% triethylamine 0.5% in water (pH adjusted to 2.5 with H3PO4) at a flow rate of 0.5 ml/min. Detection of Levofloxacin was done at 292 nm, and the column temperature was 40 °C[44].

Ghimire S *et al*, presented an LC-MS method for the estimation of Levofloxacin and its metabolite (desmethyl-Levofloxacin) in human serum. Sample preparation was achieved by using protein precipitation technique and gradient elution utilizing ultra-pure water, acetonitrile and an aqueous buffer (containing ammonium acetate 5.0 g/L, 100% acetic acid 35 mL/L and trifluoroacetic acid 2 mL/L in water. HPLC system was coupled to a quadruple mass spectrometer. Quantifications were achieved in selected reaction monitoring mode and electrospray ionization was operated in positive mode with a spray voltage of 3500 V[45].

HPLC-ESI-MS/MS method for the determination of Levofloxacin and ciprofloxacin concentrations in human prostate bioptates was developed using RP C18 column in the isocratic elution mode within 3.5 min run time. The mobile phase consisted of 0.1% formic acid aqueous solution and 0.1% formic acid methanol solution (79:21, v/v)[46].

Another HPLC method with ultraviolet (UV) detection for Levofloxacin in patients on MDR-TB treatment was based on a solid phase extraction (SPE) and a direct injection into the HPLC system employing a C18 column at 30C using a gradiant mobile phase of continuous acetonitrile 5 ± 75% with 10mM-monobasic potassium phosphate of pH 3.5 (1.0 mL/min) and detection at 295 nm[47].

Patel Dhara *et al*, developed a stability indicating RP-HPLC method for the simultaneous determination of Cefixime trihydrate and Levofloxacin hemihydrate in pharmaceutical formulations. This method was achieved on C18 (250 × 4.6 mm × 5 μ) column at a flow rate of 1 mL/min utilizing a mobile phase composed of 0.5% Glacial acetic acid in water pH adjusted to 4.5 with ammonia solution: Methanol (45:55% v/v) and eluents were monitored using PDA detector at 290 nm[48].

Another RP-HPLC was developed on C18 column (250, 4.6 mm, 5 ml) with ultraviolet detection at 306 nm for the determination of Levofloxacin Hemihydrate and ambroxol hydrochloride in bulk and pharmaceutical dosage forms using a mixture of acetonitrile, potassium dihydrogen phosphate (pH 5, KH_2PO_4), and methanol (25:65:10)[49].

RP-HPLC method was performed for the quantitative determination of Levofloxacin in bulk material, pharmaceutical formulation and serum using C18 (25 cm × 4.6 mm, 5 μm) column and the mobile phase was methanol: water (70:30, v/v) eluted at a flow rate of 1 ml/min[50].

Isocratic RP-HPLC was achieved on a C18 (250 × 4.6 mm, 5 μm) column using acetonitrile-methanol-phosphate buffer 0.1 M in the ratio 15:25:60 (v/v/v) as a mobile phase with flow rate of 1 ml/min. UV detection was carried out at 287 nm and fluorometric detection at excitation/emission wavelengths of 300/500 nm[51].

LC-MS/MS method was presented for the determination of Levofloxacin in human plasma. The separation was achieved on a Zorbax SB-C18 column under isocratic conditions using a mobile phase of 17:83 (v/v) acetonitrile and 0.1% (v/v) formic acid in water at 50 °C at a flow rate 1 mL/min and the detection was performed in multiple reaction monitoring mode using an ion trap mass spectrometer with electrospray positive ionization[52].

Isocratic, reversed phase-liquid-chromatographic method for the quantitative determination of azithromycin and Levofloxacin in combined-dosage form was achieved on RP18, (250 × 4.6, 5 μ) column with a mobile phase consisted of water pH 9.2 adjusted with di- potassium hydrogen phosphate: methanol in the ratio of (60: 40, v/v) with a flow rate 1.0 mL/min, column temperature was kept at 30 °C and effluents were measured at 285 nm[53].

Belal *et al*, presented micellar HPLC method for the simultaneous determination of Levofloxacin and ambroxol in their combined dosage forms and plasma using C18 column (150 mm × 4.6 mm i.d., 5 μm) and mobile phase composed of 0.15 M sodium dodecyl sulfate, 8% n-propanol, 0.3% triethyl amine, prepared in 0.02 M orthophosphoric acid adjusted at pH 4.0 utilizing orthophosphoric acid and the flow rate was at 1 mL/min. The column was operated at ambient temperature and the wavelength was measured at 220 nm[54].

HPLC method with ultraviolet detection was developed for the quantification of Levofloxacin in human plasma utilizing a protein precipitation technique and a short chromatographic running time (4.5 min). The separation was achieved on a Symmetry C18 column using a mobile phase consisted of acetonitrile and 0.01 M potassium dihydrogen aqueous solution (pH 3.4; 14:86 v/v)[55].

Another RP-HPLC method for the analysis of Levofloxacin and ornidazole was developed using a Hypersil BDS C18 150 mm × 4.6 mm × 5 μm column in isocratic mode, with a mobile phase composed of buffer: acetoni-

trile (75:25) at a flow rate 1.0 ml/min and the detector was set at 315 nm[56].

HPLC method was developed for the determination of Levofloxacin from human plasma. Levofloxacin was extracted with ethyl acetate from plasma and eluted by 80:20 v/v phosphate buffer pH 2.5, acetonitrile on C18 column (4.6 × 250 mm × 5 μm) at a flow rate of 1 ml/min with UV detection at 235 nm[57].

HPLCMS/MS method for the simultaneous estimation of isoniazid, rifampicin and Levofloxacin in mouse tissues and plasma was presented using gatifloxacin as the internal standard (I.S.). The compounds and I.S were extracted from tissue homogenate and plasma by a protein precipitation with methanol. The separation was carried out on a C4 column (250 mm × 4.6 mm, 5.0 μm) at 25 °C, using a gradient elution program with the initial mobile phase consisting of 0.05% formic acid and methanol (93:7, v/v) with flow-rate of 1.0 ml/min[58].

HPLC method with fluorescence detection for the determination of Levofloxacin in human plasma was described. Levofloxacin and terazosin (internal standard, IS) were separated on a C18 column using a mobile phase consisting of phosphate buffer (pH 3.0, 10 mM), acetonitrile and triethylamine in the ratio (76:24:0.076, v/v/v) at a flow rate 1 mL/min. The eluents were determined using fluorescence detection at an excitation and emission wavelength of 295 and 440 nm, respectively[59].

RP-HPLC was described for the determination of seven quinolones in plasma and amniotic fluid. The experimental responses were fitted into a second order polynomia. The optimum assay conditions were: 15 mM citrate buffer, pH 3.2, 9% ACN, 5% MeOH, 5 mM TMAB, 1.5 mL/min flow rate and 40 °C column temperature using solid phase extraction for the preparation of samples[60].

C18 column (150 × 4.6 mm i.d., particle size 4 mm) was utilized for determination of Levofloxacin under isocratic elution using a mobile phase containing water: acetonitrile: phosphoric acid 0.025 M, pH adjusted to 3.0 with trimethylamine in the ratio (60:20:20, v/v/v), at ambient temperature and at a flow rate 1.0 mL/min. The UV detection was at 294 nm and UV Vis spectrophotometer at 292 nm[61].

HPTLC method was developed for the analysis of Levofloxacin in pharmaceutical formulations utilizing silica gel 60 F254 prewashed with methanol; water-methanol-n-butanol-ammonia solution (5:5:5:0.4, v/v/v/v) was used as mobile phase. Detection and quantification were performed densitometrically at λ = 298 nm[62].

S.Siewert presented HPLC method coupled with fluorescence detection for the estimation of Levofloxacin in plasma and dialysate. Samples were prepared by one-step protein precipitation for plasma or direct injection of the dialysate solution, respectively. Separation was achieved on an YMC Pro C18 RP column (150 mm × 2 mm) with an acidic binary gradient mobile phase and detection was at excitation and emission wavelengths of 296 and 504 nm[63].

HPLC method was reported for the quantification of Levofloxacin in human plasma, bronchoalveolar lavage and bone tissues. The method used UV detection at 299 nm and a separation was achieved with a Supelcosil ABZ+ column after sample extraction that was based on a fully automated liquid-solid extraction with an OASIS cartridge[64].

RP-HPLC was developed for the determination of Levofloxacin and ciprofloxacin simultaneously in microdialysis and plasma samples using fluorescence detection. After sample preparation, the analytes were eluted in the isocratic mode within 12 min[65].

HPLC method for the determination Levofloxacin in serum, bile, soft tissue and bone was developed. The samples were prepared by protein precipitation with acids and methanol, yielding high recoveries (for serum and bile $>$ 98% and for bone and soft tissue $>$ 90%). The separation was carried out on a reversed phase column with a mobile phase consisting of triethylamine using a fluorescence detection[66].

3.1.3 Spectrophotometric methods

Zero order derivative spectroscopic method was described for the dtermination of Levofloxacin in bulk and pharmaceutical formulations with maximum absorption at 293.6 nm in 0.1N sulphuric acid[67].

Spectrophotometric method for the determination of ciprofloxacin and Levofloxacin was performed in pure form and pharmaceutical tablets. Both drugs gave reddish complexes when treated with iron (III) chloride at pH 4.0 showing a maximum absorption at 530 and 545 nm[68].

UV-Visible spectrophotometric method for the estimation of Levofloxacin in bulk and pharmaceutical formulation was developed using 0.1N NaOH as a solvent and absorption maximum (λ max) of the drug was found to be 288 nm[69].

UV spectrophotometric method was presented using a solvent containing water: methanol: acetonitrile in the ratio of (9:0.5:0.5 v,v) to estimate the Levofloxacin in bulk and pharmaceutical formulations. At a pre-determined λ max of 292 nm[70].

Desai *et al*, developed UV spectrophotometric method for the determination of Levofloxacin in dosage formulations at a pre-determined λ max of 290 nm with 0.1M HCl as diluent/blank[71].

3.1.4 Miscellaneous methods

A glassy carbon electrode (GCE) was modified with a nanocomposite prepared from polymerized β-

cyclodextrin (β-CD) and reduced graphene oxide (rGO) for the voltammetric determination of traces of Levofloxacin in human plasma by various electrochemical techniques[72].

Another voltammetric method for the determination of Levofloxacin using glassy carbon electrode (GCE) which were modified using reduced graphene oxide (rGO). The behavior of the modified GCE (mGCE) were assessed based on cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and UV-Vis spectroscopy, and its surface was studied through scanning electron microscopy (SEM) tests. Electrochemical measurements were performed using the electrode in differential pulse voltammetry (DPV) and CV experiments[73].

Chaoqiao Liu *et al*, described a simplified chemical precipitation method and silver mirror reaction to synthesize a nanocomposite consisting of silver nanoparticles on a thin and porous nickel oxide film. Placed on a glassy carbon electrode (GCE), it allows for the determination of Levofloxacin (LEV) via square wave voltammetry (SWV)[74].

Multivariate strategy was utilized for developing a fluorescent technique for the estimation of norfloxacin, Levofloxacin and lomefloxacin in their pure and dosage forms. Based on their known interaction with lanthanides, and augmented fluorescence intensity obtained by antenna effect at λex/λem=314/553, 312/553 and 310/556 for NOR, LEV and LOM respectively[75].

Spectrofluorometric method was developed for the analysis of 3 Fluroquinolones (FQ) named enrofloxacin, Levofloxacin and ofloxacin in pharmaceutical formulations by charge transfer (CT) complex formation using 2,3,5,6-tetrachloro-p-benzoquinone (chloranil, CLA). The FQ-CLA complexes showed excitation maxima ranging from 359 to 363 nm and emission maxima ranging from 442 to 48 8nm[76].

4 Vancomycin

Vancomycin[5] as Figure 3 shows.

Molecular formula: $C_{66}H_{75}C_{12}N_9O_{24}$

Molecular weight: 1449.3 g/mol

Chemical name: (3S, 6R, 7R, 22R, 23S, 26S, 30aSa, 36R, 38aR)-3-(2-amino-2-oxoethyl)-44[[2-O-(3-amino-2, 3, 6-trideoxy-3-C-methyl-α-L-lyxo-hexopyranosyl)-β-D-glucopyranosyl] oxy]-10, 19-dicholoro-7, 22, 28, 30, 32-pentahydroxy-6-[[(2R)-4-methyl-2-(methylamino) pentanoyl] amino]- 2,5,24,38,39-pentaoxo-2, 3, 4, 5, 6, 7, 23, 24, 25, 26, 36, 37, 38, 38a-tertadecahydro-22H-8, 11:18, 21-dietheno-23, 36-(iminomethano)- 13, 16:31, 35-dimetheno-1H,13H-[1, 6, 9] oxadiazacy clohexadecino[4, 5-m][10, 2, 16] benzoxadiazacyclo tetracosine - 26- carboxylic acid

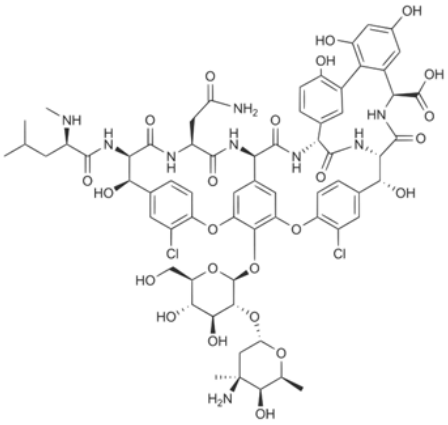

Figure 3. Levofloxacin

Physical properties: It is official drug in USP[6] and BP. It is a white, hygroscopic powder. Freely soluble in water; slightly soluble in alcohol. Store in air-tight containers. Protect from light[5].

Melting point: Around 190°C.

Mechanism of action: Vancomycin has a glycopeptide structure; it acts by interfering with bacterial cell wall synthesis through inhibiting the formation of the peptidoglycan polymers. It is very active against Gram-positive cocci including MRSA[7].

Uses and administration: It is used particularly in the treatment of methicillin-resistant staphylococcal infections in hospital acquired pneumonia with a dose 15 mg/kg IV q812h with goal to target 15-20 mg/mL trough level (consider a loading dose of 2530 mg/kg $\times$ 1 for severe illness) and other conditions such as brain abscess, staphylococcal meningitis, peritonitis associated with continuous ambulatory peritoneal dialysis, and septicaemia[7].

4.1 Methods of determination

4.1.1 Pharmacopeal method

BP: Vancomycin was determined by microbiological assay of antibiotics using Vancomycin as the chemical reference structure[5].

USP: as mentioned above in BP[6].

4.1.2 Chromatographic methods

LC-MS/MS assay was developed for the simultaneous determination of Vancomycin and creatinine in plasma applied to volumetric absorptive microsampling devices (VAMS). Estimation approaches were used for the measurements of Vancomycin and creatinine as their concentrations measured in VAMS differ from those in plasma[77].

An UPLC-MS/MS method was performed for the determination of Vancomycin in plasma by minimizing the

interference of crystalline degradation product and matrix[78].

The pharmacokinetics and tissue residues of Vancomycin in crucian carp (Carassius auratus) were studied after oral administration by an LC-MS/MS system coupled with quantum ultra triple-quadrupole mass spectrometer with electrospray ionization (ESI) source. Separation was achieved on a C18 (2.0 × 100 mm, 5 μm) column by gradient elution using a mobile phase composed of mixture of eluent A (acetonitrile) and eluent B (water containing 0.1% formic acid) at a flow rate 0.3 ml/min and the column was maintained at 35 ∘C[79].

UHPLC-Triple-TOF-MS/MS method in positive ion mode was developed for studying Vancomycin metabolism in vitro and its natural degradation in aquatic environment. This method was achieved on EC-C18 (2.1 × 150 mm, 2.7 μm) column maintained at 40 °C using the mobile phases A (0.1% aqueous formic acid) and B (acetonitrile) to optimize the gradient elution program at a constant flow rate 0.4 mL/min[80].

High-performance liquid chromatography - tandem mass spectrometry (Q-trap-MS) method was presented for the determination of Vancomycin in human serum using norvancomycin as an internal standard. ZORBAX SB-C18 column (150 × 4.6 mm, 5 μm) was used with a mobile phase composed of water (containing 0.1% formic acid, v/v)acetonitrile (containing 0.1% formic acid, v/v)[81].

HPLC method using core shell particles for the determination of Vancomycin in human plasma is reported with chemometric optimization. Separation was achieved on C18 (3.0 mm × 75 mm, 2.7 μm) column utilizing a mixture of 50 mmol/L phosphate buffer solution and MeOH as a mobile phase[82].

A LC-MS/MS method was developed using hydrophilic interaction liquid chromatography for the estimation of Vancomycin in plasma (total, unbound), urine and renal replacement therapy effluent[83].

High performance liquid chromatography was achieved isocratically by using a mobile phase $NH_4H_2PO_4$ (50 mM, pH 2.2)acetonitrile (88:12, v/v) with a flow rate at 0.36 mL/min eluted on a nucleodur C18 column (125 mm × 4.6 mm, 5 μm). UV detection was set at 205 nm after sample preparation by deproteination of plasma with 70% perchloric acid and a liquid/liquid extraction[84].

High performance liquid chromatography with diode array detection (DAD) for the quantification of Vancomycin in human plasma by solid phase extraction with isolute-cartridges C8, IST (50 mg/mL) was carried out on Poroshell 120, C8 EC, 2.7 μm, 100 mm × 4.6 mm i.d utilizing a mobile phase composed of potassium dihydrogen phosphate buffer 25 mM, pH 3.0 acetonitrile in the ratio of (92:8, v/v) with a flow rate of 1mL/min at 40 °C[85].

A LCMS/MS Analysis was performed on an Acquity TQD mass spectrometer equipped with an Acquity UPLC 2795 separations module for the determination of Vancomycin in plasma and compared with immunoassays[86].

An HPLC method was performed on Nucleosil 120 C18 5 μm column at 35 °C with mobile phase containing acetonitrile (A): water, pH 2.0 (B) and formic acid was used to adjust the pH of water at a flow rate of 1 mL/min[87].

LC-MS/MS assay for the determination of Vancomycin in human plasma, bone and fat tissue was achieved on a C18 (2) 5 μm, 50 × 2.0 mm analytical column equipped with a C18 4.0 × 2.0 mm guard column using gradient elution of 0.05% formic acid and methanol and were detected using electrospray ionisation in the positive mode[88].

UPLCTQD method was developed for the determination of tobramycin and Vancomycin in osteomyelitis patient samples on a C18 column (2.1 × 100 mm, 1.7 mm) with a mobile phase containing 0.1% (v/v) formic acid water solution and (0.1% v/v) formic acid acetonitrile solution at a flow rate of 0.3 mL/ min and the detection was carried out by positive ion electrospray ionization in multiple reaction monitoring mode (m/z 468 →163 transitions for tobramycin; m/z 725→144 for Vancomycin[89].

HPLC method with an UV detector set at 240 nm utilizing a protein precipitation technique, was developed to analyze Vancomycin in human plasma, mouse serum and bronchoalveolar lavage fluid. Separation was achieved on C18 ODS column (4.6 × 150 mm) coupled to a μ Bondapak C18 10 mm Guard-pak precolumn with a mobile phase consisted of a mixture of 0.05M ammonium phosphate buffer with 11% acetonitrile[90].

Determination of Vancomycin content in human plasma was done by LC-MS/MS method, where separation was achieved on a Shimadzu VP-ODS column (2.0 mm × 150 mm, 5 μm) with a mobile phase containing 0.1% formic acid in water and acetonitrile. The mobile phase was gradient eluted at a flow rate of 0.3 mL/min[91].

An ultra high-pressure liquid chromatographic (UHPLC) method, was used to evaluate Vancomycin products using C18 column (100 by 2.1 mm, 1.7 μm) operating at 40 °C, separation was carried out using a binary gradient where mobile phase A was 95:5 (vol/vol) H_2O-0.2 M ammonium acetate, pH 9, and mobile phase B was 65:30:5 (vol/vol/vol) H_2O-methanol-0.2 M ammonium acetate, pH 9 at a flow rate was 200 μl/min[92].

High performance liquid chromatography method was reported for the determination of Vancomycin concentrations in plasma and pig pulmonary tissue using a silica based column Symmetry 300 C18 (150 × 4.6 mm) with pre-column. The mobile phase composed of 20% ultra-

filtered water and 80% of (A) 75 mM sodium acetate buffer (pH = 3) with (B) acetonitrile (92%/8%; v/v) with a flow rate at 0.8 mL/min and 0.7 mL/min for plasma and tissue samples, respectively. UV detection was carried out at 230 nm[93].

UHPLC-UV method for the simultaneous estimation of furosemide, saluamine (furosemide metabolite), spironolactone, carnenone (spironolactone active metabolite), terbinafine, N-desmethylcarboxy terbinafine (terbinafine metabolite) and Vancomycin in human plasma and urine was presented using a Hypersil GOLD C_{18e} (50 mm × 2.1 mm, 1.7 μm particles) with the mobile phase composed as acetonitrile and 0.1% formic acid. The determined substances were eluted from column in 3.3 min[94].

RP-HPLC method with UV detection at 280 nm was reported for the simultaneous quantification of ceftriaxone sodium and Vancomycin Hydrochloride. Where the separation was carried out on a Betasil C-1 column using a mobile phase composed of acetonitrile and triethylamine buffer adjusted to pH 3.5 4 ± 0.1 with orthophosphoric acid in a ratio of 20:80[95].

HPLC method with fluorescence detection for the determination of Vancomycin in human plasma was described. Extraction of Vancomycin was performed by deproteinization with acetonitrile. The analyses was carried out at 258 nm as the emission wavelength while exciting at 225 nm on a RP C18 column (30 cm × 4 mm i.d. × 10 μm) using a mobile phase composed of methanol and phosphate buffer at pH 6.3[96].

HPLC Separation was achieved on a diamonsil C18 column (200 mm × 4.6 mm I.D., 5 microm) using a mobile phase composed of acetonitrile and acetate buffer (pH 3.5) (8:92, v/v) at room temperature (25 °C), and the detection wavelength was 240 nm for the determination of Vancomycin and ceftazidime in cerebrospinal fluid in craniotomy patients[97].

RP-HPLC was carried out by discovery RP amide C16 (4.6 mm × 250 mm 5 μm) column with mobile phase composed of acetonitrile-0.05 mol/L potassium dihydrogen phosphate buffer solution (10:90, pH=2.8). The flow rate was 1.0 mL·min-1.The detection wavelength was 230 nm[98].

An automated analyzer for Vancomycin in rat plasma by column-switching high-performance liquid chromatography (HPLC) with UV detection was developed. The mobile phase for pre-treatment was 67 mm phosphate buffer (pH 5.3) and the flow rate was 1.0 mL/min. The mobile phase for separation was 50 mm phosphate buffer) (pH 6.75)/acetonitrile (88/12, v/v) and the flow rate was 1.0 mL/min[99].

Another HPLC with electrochemical detection for the analysis of Vancomycin in human plasma was developed. Chromatographic separation was achieved on an octadecyl column using a mobile phase of acetonitrile-sodium phosphate buffer (pH 7)(12:88 v/v) within 12 min, and coulometric electrochemical detection at +700 mV[100].

4.1.3 Spectrophotometric methods

UV spectrophotometric method was developed for the determination of Vancomycin Hydrochloride in pharmaceutical preparations that showed maximum absorbance at a wavelength of 281 nm in phosphate buffer pH 6.8[101].

Batch and flow-injection spectrophotometric methods for the estimation of Vancomycin Hydrochloride in pure form and in pharmaceutical formulations were developed. These methods were based on diazotization and coupling reaction between Vancomycin Hydrochloride and diazotized Procaine penicillin in alkaline medium to form an intense yellow water-soluble dye that is stable and has a maximum absorption at 446 nm[102].

Another UV- spectrophotometric method was presented for the determination of Vancomycin in human plasma using ultracentrifuge protein precipitation[103].

Hind Hadi developed two UV methods for the quantitative estimation of Vancomycin Hydrochloride and its formulations based on a coupling reaction between Vancomycin and diazotized procain (method A) or diazotized sulphacetamide sodium (method B) in alkaline medium to form intense yellow, water-soluble dyes that are very stable and have a maximum absorption at 447 and 439 nm for methods A and B respectively[104].

Another spectrophotometric method for the determination of Vancomycin base, and Vancomycin Hydrochloride, based on the reaction with copper (II) ions, was presented[105].

A.M. El-Didamony *et al*, performed four procedures for the determination of Gentamicin Sulphate and Vancomycin Hydrochloride, both in pure form and in pharmaceutical dosage forms. The methods are based on the oxidation of the studied drugs by a known excess of potassium permanganate in sulphuric acid medium and subsequent determination of unreacted oxidant by reacting it with amaranth dye (method A), acid orange II (method B), indigocarmine (method C) and methylene blue (method D), in the same acid medium at a suitable λ max = 521, 485, 610 and 664 nm, respectively[106].

4.1.4 Miscellaneous methods

Electroanalytical determination of Vancomycin at a graphene-modified electrode using square wave voltammetry was developed for the determination of Vancomycin in human plasma[107].

Specific spectrofluorimetric method was developed and validated. Vancomycin fluorescence was measured at 335 nm when excited at 268 nm where Vancomycin stability was studied when exposed to various degradation

conditions such as oxidative, alkaline as well as acidic stress[108].

A competitive immunoassay was developed for Vancomycin using capillary electrophoresis with laser-induced fluorescence. The antibody-bound was separated from the unbound fluorescent probe (tracer) by the capillary electrophoresis in less than 4 min using a tris-glycine running buffer. The fluorescence polarization value was 0.30[109].

Two methods are described for the determination of Vancomycin Hydrochloride in pharmaceutical dosage forms. The two methods included a prior treatment with nitrous acid then measuring the formed nitroso derivative, either spectrophotometrically or polarographically[110].

5 Gentamicin

Gentamicin[5] as Figure 4 shows.

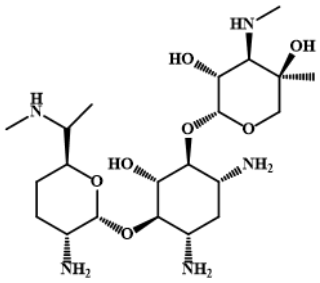

Figure 4. Levofloxacin

Molecular formula: $C_{21}H_{43}N_5O_7$

Molecular weight: 477.6 g/mol

Chemical name: 2-[4,6-diamino-3-[3-amino-6- [1-(methylamino) ethyl] oxan -2 -yl] oxy-2 -hydr oxy cycl ohexyl] oxy- 5- methyl- 4-(methylamino) oxane-3,5-diol

Physical properties: It is an official drug in USP[6] and BP. A white or almost white hygroscopic powder. Freely soluble in water; insoluble in alcohol, in acetone, in chloroform, in ether, and in benzene. Store in air tight containers.

Melting point: 218-237°C

Mechanism of action: It is broad-spectrum bactericidal aminoglycosides with activity against many aerobic Gram negative and some aerobic Gram-positive organisms, but it is typically used for moderate to severe Gram-negative infections; it acts by binding to bacterial ribosomes and inhibiting protein synthesis[7].

Uses and administration: Gentamicin is used, often with other antibacterials, to treat severe systemic infections such as endocarditis (in the treatment and prophylaxis of endocarditis due to streptococci, enterococci, or staphylococci), endometritis, pneumonia, septicemia and many other infections.

In the prophylaxis and treatment of streptococcal and enterococcal endocarditis, a dose of 1 mg/kg every 8 hours with a penicillin or Vancomycin is used[7].

5.1 Methods of determination

5.1.1 Pharmacopeal method

BP: Microbiological assay of antibiotics[5].

USP: Also by microbiological assay[6].

5.1.2 Chromatographic methods

C18 column (50 mm × 4.6 mm, 5 μm) with mobile phase consisting of Buffer (N- Heptane Sulphonic acid): MeOH: GAA (125:350:2.5) were used for the determination of Gentamicin at a flow rate 0.2 ml/ min and detection at 330 nm[111].

A stability indicating HPLC assay with UV detection for simultaneous quantification of Gentamicin Sulfate and EDTA-Na_2 was developed while antibiotic lock solution was determined by ion-pairing high performance liquid chromatography (HPLC) needing Gentamicin derivatization, EDTA-Na2 metallocomplexation of samples and gradient mobile phase[112].

LC-MS/MS method with simple sample extraction and a relatively short time of Gentamicin analysis was developed. 50 μL of serum was precipitated using acetonitrile and formic acid and A RP BEH C18, 1.7 μm, 2.1 × 50 mm column was maintained at 30 °C with mass detection in positive electrospray mode[113].

High performance liquid chromatography with evaporative light scattering was performed for the simultaneous detection of ten aminoglycoside antibiotics including apramycin, neomycin, amikacin, and Gentamicin using a Hypersil BDS C18 column based on ion-pair chromatography[114].

LC-MS/MS was developed for total Gentamicin quantification to be used for routine assays applied for therapeutic drug monitoring of this compound. Sample preparation was based on sample dilution with an aqueous internal standard solution followed by protein precipitation[115].

HPLC method for quantitative analysis of Gentamicin was developed using a quality by design (QbD) a statistical approach. Chromatographic separation was achieved on a C- 18 column (250 × 4.6 mm, 5 micron) using isocratic elution system composed of methanol and 15 mM diammonium hydrogen phosphate buffer (pH 10.00) in the ratio 70:30 v/v[116].

A RP-HPLC method was developed for the determination of Gentamycin Sulfate in poultry meat. Samples were prepared by an extraction with phosphate buffer/TCA followed by a solid phase cleanup and then UV detector. The separation was carried out on C18 (4.6 mm i.d., 250 mm, 5 μm, Agilent Co.) column and the isocratic mobile

phase containing (0.1M) of triflouroacetic acid: methanol (92:8v/v) was used at a column temperature of 24 C at a flow rate 1.5 ml/min. Detection was carried out at 280[117].

A RP-HPLC method linked to the mass Spectrometry (MS), using the pattern of electrospray ionization source in the positive ion mode, was developed for the determination of Gentamicin Sulfate and its impurities in injectable dosage form using a reverse phase C18 column and 50 mM trifluoroacetic acid, pH 2 adjusted with ammonium solution, as mobile phase at a flow rate of 0.25 mL /min[118].

LC-MS/MS method for the separation, detection and quantification of 12 aminoglycosides using two chromatographic conditions (HILIC, Ion pairing). The comparison between the two separation methods shows that the response area of the majority of analytes tested increases when using the ion pair mode[119].

Three LC columns were selected: (1) AquaO C18, 5 micron, (2) LunaO C18, 5 micron, and (3) Nuc leosilO C18, 3 micron (all in 4.6 × 150 mm) to evaluate the performance of different C18 columns for separating the aminoglycosides in the USP reference standard and samples prepared from a Gentamicin Sulfate powder met the USP test specifications[120].

Reversed phase HPLC and microbiological assay were developed for the determination of Gentamicin, Gentamicin concentrations measured by the agar well method using staphylococuss epidermidis (NCIM 2493) and bacillus pumilus (NCIM 2327) as test organisms[121].

LC equipped with ion trap MS with positive ionization was performed on a Capcell Pak C18 (AQ) column with the mobile phase containing 50 mM trifluoroacetic (TFA) and methanol for the determination of Gentamicin in bulk samples[122].

High-speed counter-current chromatography coupled with mass spectrometry (HSCCC/MS) to purify Gentamicin C1a, C2/2a and C1 from standard powder. The analytes were purified on the HSCCC model CCC (multilayer coil planet centrifuge) with a volatile two-phase solvent system composed of n-butanol/10% aqueous ammonia solution (50:50, v/v) and detected on an LCMS-2020 EV quadrupole mass spectrometer fitted with an electrospray ionization source system in positive ionization following scan mode (m/z 100500)[123].

A reversed phase HPLC using Zorbax Rx-C-18 2.1 × 150 mm, 5 μm column with a mobile phase consisted of methanol-water-acetate buffer (0.02 M ammonium acetate solution, adjusted with cc. ammonia to pH = 9 were used for the determination Gentamicin released from orthopedic carrier system[124].

Liquid chromatography-tandem mass spectrometry was reported for the determination of dihydrostreptomycin, streptomycin, apramycin, neomycin and Gentamicin (C1, C2 and C1a) present in animal tissue and dihydrostreptomycin, streptomycin, neomycin and Gentamicin (C1, C2 and C1a) present in bovine milk utilizing a 5 μm C18 HPLC column and a mobile phase composed of acetonitrile, water and 50 mM heptafluorobutyric acid[125].

HPLC-MS quantification of daptomycin, amikacin, Gentamicin, and rifampicin in human plasma involved a rapid sample preparation by protein precipitation with acetonitrile followed by direct injection[126].

RP-HPLC method to determine the composition of Gentamicin sulfate and to estimate its related substances was achieved on a short (5 cm × 4.6 mm) pentafluorophenyl HPLC column (Allure PFP) using an ion-pair gradient mobile phase consisting of (A) heptafluorobutyric acid:water:acetonitrile (0.025:95:5, v/v/v) and (B) trifluoroacetic acid:water:acetonitrile (1:95:5, v/v/v)[127].

High-performance liquid chromatography-mass spectrometric method was performed for the determination of Poly (lactide-co-glycolide) microparticles (MP) of Gentamicin (PLGA 502H MP), THP-1 cells, and plasma and tissue samples of mice treated with the antibiotic either free or loaded into PLGA 502HMP after being processed by a simple preparation procedure[128].

LC technique employed trifluoroacetic acid in the mobile phase so that all Gentamicin components co-eluted. Analytes were ionized by positive-ion pneumatically assisted electrospray and detected by selected reaction monitoring (SRM) with an LC-tandem mass spectrometer (LC/MS/MS)[129].

HPLC method for determination of Gentamicin Sulfate and neomycin sulfate using 9-fluorenylmethyl chloroformate for derivatization was achieved on an agilent C18 column (15 0mm × 4.6 mm, 5μm) as stationary phase with acetonitrile-water (955, V/V) as mobile phase, the detection wavelength was 265 nm at a flow rate 1 mL/min, and the temperature of column was 25 °C.[130].

High performance liquid chromatography with evaporative light scattering detection for the separation and quantitation of Gentamicin Sulfate. Separation was performed on a high purity C18 125 mm × 4 mm i.d., 3 μm, reversed phase column with 48.5 mM trifluoroacetic acidmethanol (97:3, v/v), as mobile phase at a flow rate of 0.7 ml/min[131].

Three components of Gentamicin C1, C1a, and C2 in plasma and urine were isolated by preparative chromatography and verified by thin-layer chromatography, HPLC, mass spectrometry, nuclear magnetic resonance spectroscopy. These 3 components were extracted from the biological matrix by use of tris buffer and polymer

phase solid-phase extraction. Derivatization was carried out in the solid-phase extraction cartridge with 1-fluoro-2, 4-dinitrobenzene forming 2, 4-dinitrophenyl derivatives that were separated with reversed-phase HPLC and quantified by the UV detection at 365 nm[132].

An HPLC method was described for the determination of Gentamicin in bacterial culture medium or plasma with increased sensitivity and improved separation of the C1 component. Gentamicin was extracted from the biological matrix with high efficiency using carboxypropyl (CBA)-bonded silica. Derivatization with 9-fluorenylmethyl chloroformate followed by C18 reversed-phase chromatography allowed the fluorimetric detection of Gentamicins C1, C1a and C2[133].

High performance liquid chromatography with mass spectrometry was performed on-line utilizing thermospray mass spectrometry (HPLC-TSP-MS) for the determination of the major components (C1a, C2 and C1) of Gentamicin sulfate as well as an additional minor component by reversed-phased separation utilizing trifluoroacetic acid as an ion pair reagent[134].

High performance liquid chromatography utilizing pre-column derivatization with UV detection was developed for the determination of Gentamicin in serum. The serum proteins are precipitated with acetonitrile and the Gentamicin components in the supernatant are derivatized with 1-fluoro-2, 4-dinitrobenzene and the reaction products are separated on a microparticulate C18 reversed-phase column with detection at 365 nm[135].

HPLC method for the determination of Gentamicin Sulphate was presented, utilizing pre-column derivatization with an o-phthalaldehyde/thioglycollic acid reagent, ion-paired chromatography of the derivative on a Hypersil O.D.S. column and UV detection[136].

5.1.3 Spectrophotometric methods

Spectrophotometric method was developed via ninhydrin-Gentamicin complex as a rapid quantification approach for the determination of Gentamicin loaded with poly (lactic-co-glycolic acid) PLGA microparticles that was suitable for Gentamicin encapsulation without compromising the efficacy of the antibiotic itself[137].

Two kinetic spectrophotometric methods for the determination of Gentamicin in pharmaceutical formulations based on the oxidation of Gentamicin drug with alkaline potassium permanganate at fixed temperature followed spectrophotometrically by measuring the increase in the absorbance owing to the formation of MnO_4^{2-} at 610 nm, based on initial-slope and fixed-time methods[138].

Another spectrophotometric technique was presented for the assay of Gentamycin based on the ultraviolet absorbance maxima at about 202 wavelength of Gentamycin using water as solvent[139].

Derivative spectrophotometric method for the determination of Gentamicin beside methyl and propyl hydroxybenzoates in injection solutions was carried out after modifying the Gentamicin molecule by reaction with o-phthalaldehyde. The obtained spectrum of product in methanol solution was converted into a third-derivative spectrum with detection at wavelength λ = 281 nm[140].

Spectrophotometric method was developed for the determination of gentamycin is using p-dimethylaminobenzalhyde in acetic acid/acetate buffer solution to form a Schiff base, a yellow complex with Gentamicin and its maximum absorption wavelength is 405 nm[141].

5.1.4 Miscellaneous methods

Enzyme linked immunosorbent assay (ELISA) procedure was presented for the determination of Gentamicin in dried blood spot samples collected from paediatric patients on Guthrie cards. Gentamicin was extracted from DBS samples by vortexing with the ELISA extraction buffer for 30 minutes[142].

Flow procedure based on multicommutation with chemiluminometric detection was reported for the estimation of Gentamicin Sulphate in pharmaceutical dosage forms based on Gentamicin's ability to inhibit the chemiluminometric reaction between luminol and hypochlorite in alkaline medium, causing a decrease in the analytical signal[143].

A procedure was presented for the estimation of Gentamicin, based on the use of the peroxyoxalate chemiluminescent system in presence of imidazole as a catalyst. The Gentamicin was derivatized with o-phthaladehyde (OPA) in order to obtain a fluorophore, which participates in the PO reaction, producing a Chemiluminescence emission proportional to the Gentamicin concentration. The method is developed by using a particular flow-injection analysis (FIA) manifold, employing sodium dodecyl sulfate (SDS) micellar medium as a carrier in order to avoid the degradation of PO in water[144].

6 Conclusion

We represent an up-to-date literature review about most of the reported analytical methods used for the determination of the mentioned antibiotics either alone or combined in pure, pharmaceutical dosage form and in biological fluids.

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