World Journal of Analytical Chemistry, 2015, Vol. 3, No. 1A, 21-32
Available online at http://pubs.sciepub.com/wjac/3/1A/5

DOI:10.12691/wjac-3-1A-5

Spectroscopic Methods for Analysis of Cephalosporins in Pharmaceutical Formulations

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Received April 19, 2015; Revised June 3, 2015; Accepted September 06, 2015

Abstract Cephalosporins are the most commonly prescribed β-lactam antibiotics. Spectrophotometry is probably the most convenient analytical technique for routine analysis, because of its inherent simplicity, low cost and wide availability in quality control laboratories. Several papers have been presented in recent years regarding the development and validation for spectrophotometry methods for analysis of cephalosporins in pharmaceutical formulations. In this review article, various spectroscopic methods for analysis of cephalosporins are presented and discussed.

Keywords: *cephalosporins, antibiotics, spectrophotometry, pharmaceutical formulations*

Cite This Article: Shazalia Mahmoud Ali, Abdalla A. Elbashir, and Hassan Y. Aboul-Enein, "Spectroscopic Methods for Analysis of Cephalosporins in Pharmaceutical Formulations." *World Journal of Analytical Chemistry*, vol. 3, no. 1A (2015): 21-32. doi: 10.12691/wjac-3-1A-5.

1. Introduction

Cephalosporins are considered to be the most commonly prescribed β-lactam antibiotics since their first semi-synthetic production from cephalosporin C, the parent compound, in the 1940s [1]. Cephalosporin are derived from the parent compound cephalosporin C; a natural antibiotic produced by a strain of the mold cephalosporium acremonium [2]. Cephalosporin compounds were first isolated from cultures of cephalosporium acremonium [3]. These cultures produced substances that were effective against Salmonella typhus, the cause of typhoid fever; as they inhibited the growth of a number of gram-positive and gram-negative bacteria [4].

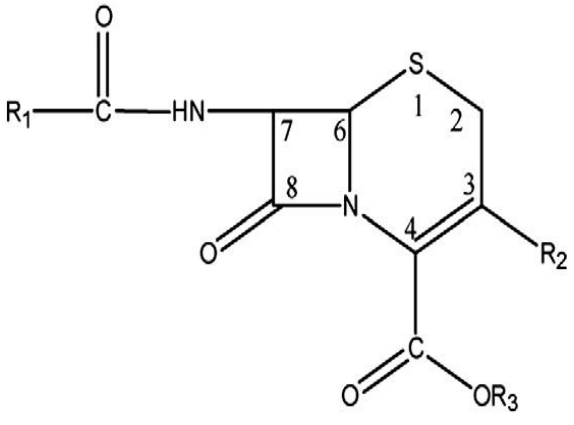


Figure 1. Chemical structure of cephalosporins

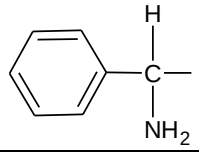
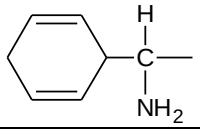
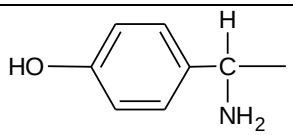
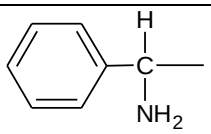
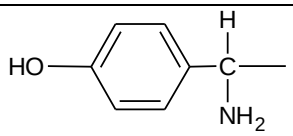
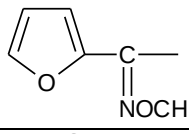
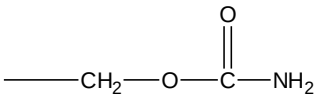
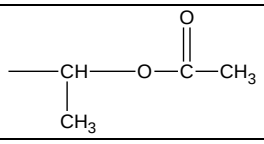
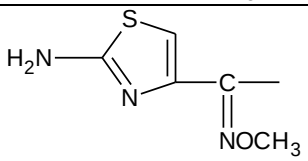
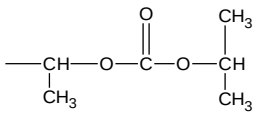
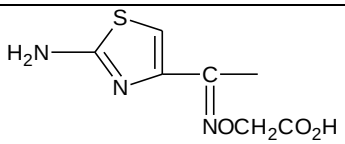
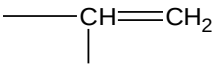
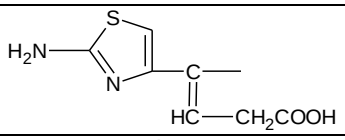
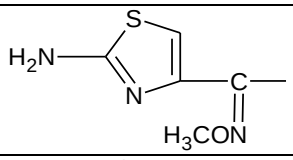
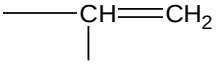
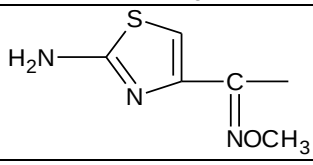
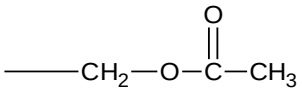
Cephalosporins are derivatives of 7-aminocephalosporanic acid (7-ACA) composed of a ß-lactam ring fused with a dihydrothaizine ring (Figure 1), but differ in the nature of substituent at C-3 (R1) and C-7 (R2) [5]. Cephalosporins resemble penicillins in that they have a ß-lactam structure, but the five-member thiazolidine ring characteristic of the penicillin is replaced by a six-member dihydrothiazine ring (Figure 1). This ring provides the molecule with the ability to resist bacterial enzymes; the antibacterial activity comes from the ß-lactam ring. Two side chains in position 3 and 7 affect the pharmacokinetic and antibacterial spectrum of the cephalosporins [6].

1.1. Classification of Cephalosporins

Cephalosporins are traditionally classified into first, second, third and fourth generation, based on the time of their discovery and their antimicrobial properties (Table 1). The first generation is resistant to the staphylococcal penicillinase. They also have activity against Proteus mirabilis, Escherichia coli, and Klebsiella pneumonia. The second generation cephalosporins display greater activity against three additional gram-negative organisms, Haemophilus influenzae, some Enterobacter aerogenes and some Neisseria species, whereas their activity against gram-positive organisms is weaker. The third generation cephalosporins have an important role in treatment of infectious disease. Though greatly inferior to first generation in regard to their activity against gram-positive cocci, they have enhanced activity against gram-negative bacilli plus most other enteric organisms and Serratia marcescens. The fourth generation cephalosporins have a

wide antibacterial spectrum being active against Streptococci and Staphylococci. They are also effective against aerobic gram-negative organisms such as Enterobacter, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis and Pseudomonas aeruginosa [7,8].

Table 1. List of chemical structures of the cephalosporins

Name	R1	R2	R3	Generation
Cephalexin		–CH_3	-H	First
Cefradine		–CH_3	-H	First
Cefadroxil		–CH_3	-H	First
Cefroxadine		–OCH_3	-H	First
Cefaclor		–Cl	-H	Second
Cefuroxime				Second
Cefpodoximeprox etil		-CH_2OCH_3		Third
Cefixime			-H	Third
Ceftibuten		-H	-H	Third
Cefetamet			-H	Third
Cefotaxime			-H	Third

1.2. Mechanism of Action of Cephalosporins

The cephalosporins, like all ß-lactams, interfere with the last step of bacterial cell wall synthesis, thus exposing the osmotic ally less stable membrane. Cell lysis can then occur, and these drugs are therefore bactericidal. Cephalosporins are only effective against rapidly growing organisms that synthesize a peptidoglycan cell wall. Consequently they are inactive against organisms devoid from this structure, such as protozoa, fungi, and viruses. Two side chains in position 3 and 7 affect the pharmacokinetic and antibacterial spectrum of the cephalosporins [9]. Pharmacokinetic properties of cephalosporin antibiotics are summarized in Table 2.

Table 2. Pharmacokinetic properties of cephalosporin antibiotics.

Drug	Generation	Half-life (min)	Half-life in ESRD (hrs)	% Protein Bound	% Unchanged in Urine
Cephalexin	First	50-80	19-22	10	>90
Cefadroxil	First	78-96	20-25	20	>90
Cephradine	First	48-80	8-15	8-17	>90
Cephalothin	First	30-50	3-15	70	68-70
Cephapirin	First	24036	1.8-4	54	68-70
Cefazolin	First	90-120	3-7	80-86	80-96
Cefuroxime	Second	80	16-22	-	-
Cefamandole	Second	30-60	8-11	70	65-85
Cefoxitin	Second	40-60	20	73	85-99
Cefonicid	Second	270	11	98	95-99
Cefixime	Third	180-240	11.5	65	50
Ceftibuten	Third	160	22.3	60-64	80-90
Cefpodoxime	Third	120	9.8	21-29	29-33
Cefoperazone	Third	102-156	1.3-2.9	82-93	20-30
Cefotaxime	Third	60	3-11	30-40	20-36

2. Analytical Methods for Analysis of Cephalosporins

Several methods have been described for the quantitative determination of cephalosporins included Spectrophotometry [10,11,12], spectrofluorimetry [13], High performance liquid chromatography (HPLC) [14-20], potentiometry [21] and voltammetry [22]. However, most of the HPLC methods were time-consuming, tedious, and dedicated to sophisticated and expensive analytical instruments. Some reviews regarding the analysis of cephalosporins were reported in the literature [23,24]. El-Shaboury 2007 [8] reported the analysis of cephalosporins antibiotics and recently Jin and co-workers 2014 [23] reviewed HPLC and LC-(MS/MS) methods for analysis of third-generation cephalosporins in biological fluids. Spectrophotometric methods are the most convenient techniques because of their inherent simplicity, high sensitivity, low cost, and wide availability in quality control laboratories. Therefore, this review was devoted to present an overview of the specrophotometric methods for analysis of cephalosporins in pharmaceutical formulations.

2.1. Spectrophotometric Methods of Analysis of Cephalosporins

El-Obeid et al., [24] reported that ampicillin, amoxicillin, cephalexin, cefadroxil and cefaclor can be determined in their pharmaceutical preparations using colorimetric method based on measuring the color obtained when the alkaline degradation product of the drug was allowed to react with ascorbic acid. Beer's law was obeyed over the range [3-15] µg mL^{-1}, and the product was measured at 410 nm.

Saleh et al., [12] described three spectrophotometric procedures for the analysis of cephapirin sodium, cefazoline sodium, cephalexin monohydrate, cefadroxil monohydrate, cefotaxime sodium, cefoperazone, and ceftazidime pentahydrate in pure form as well as in their pharmaceutical formulations. The methods were based on the reaction of these drugs as n-electron donors with the sigma-acceptor iodine, and the pi-acceptors: 2,3-dichloro-5,6-dicyano-p-benzo-quinone (DDQ) and 7,7,8,8-tetracyanoquinodimethane (TCNQ). Different variables and parameters affecting the reactions were studied and optimized. The obtained charge-transfer complexes were measured at 364 nm for iodine (in 1,2-dichloroethane), 460 nm for DDQ (in methanol) and 843 nm for TCNQ (in acetonitrile). Beer's plots were obeyed in a general concentration range of 6-50, 40-300 and 4-24 µg mL^{-1} with iodine, DDQ and TCNQ, respectively.

Pedroso and co-workers [25] developed a spectrophotometric method for the analysis of cefazolin sodium in pharmaceutical form powder for injectable solution. The reported method was capable to detect and quantify the drug and thus satisfactory results regarding specificity, precision, accuracy and robustness were obtained. Linearity range of 8-28 µg mL^{-1} with correlation coefficient of 0.9999 was obtained as well when analyzed at wavelength $\lambda = 270$ nm.

Saleh and co- workers [26] developed a kinetic spectrophotometric method for the determination of cefoperazone sodium, cefazolin sodium and ceftriaxone sodium in bulk and in pharmaceutical formulations. The method was based upon a kinetic investigation of the reaction of the drug with oxidized quercetin reagent at room temperature for a fixed time of 30 min. The decrease in absorbance after the addition of the drug was measured at 510 nm. The absorbance concentration plot was rectilinear over the range 80-400 µg mL^{-1} for all drugs studied.

Game et al., [27] developed two spectrophotometric methods for the determination of cefuroxime axetil in bulk drug and tablet. In the first method, the UV spectrum of cefuroxime axetil in 0.1N HCl was obtained at 281 nm and Beer's law was obeyed over the range 2-30 µg mL^{-1}. Method II is the 1st derivative spectrophotometric method. In this method the simple UV spectrum of cefuroxime axetil in 0.1 N NaOH was obtained and derivatized to first order. Maxima occur at 266 nm and minima at 300 nm, Beer's law was obeyed over the range 4-30 µg mL^{-1}.

Sastry et al., [28] reported a method for the analysis of cefadroxil and ceftezoxime in pharmaceutical formulations. The method was based on the addition of sodium hydroxide followed by iodine and wool fast blue and the absorbance was measured at 540 nm. The linear range was between 0.8-9.9 µg mL^{-1}.

Prayanka and Suresh [29] proposed a method for the estimation of cephalexin in capsules. This method was based on the reaction of the drug with ferric chloride and potassium ferricyanide, giving a green-colored chromogen exhibiting maximum absorbance at 791 nm. Beer's law was obeyed in the concentration range of 1-6 µg mL^{-1}.

Sayed et al., [30] developed an extractive spectrophotometric method for the estimation of gatifloxacin and cefotaxime sodium in both pure and

pharmaceutical dosage forms. The method was based on the formation of colored complex by the drugs with eriochrome black-T in an acidic medium. The ion-associated complex formed was quantitatively extracted under the experimental conditions with methylene chloride and the absorbance of the organic layer was measured at 510 nm. Beer's law was obeyed in the concentration range of 3-18 and 30-120 µg mL^{-1} for gatifloxacin and cefotaxime sodium, respectively.

Medikondu et al., [31] proposed three methods for the determination of cephalexin in bulk drug and in formulations. The methods based on redox / complex formation reaction between cephalexin and ammonium molybdate and sulphuric acid (AM/H_2SO_4) for the first method, ferric chloride/ orthophenonthraline for the second method and ferric chloride /potassium ferricyanide solutions for the third method. The chromogens being extractable with chloroform were measured quantitatively at 625, 510 and 740 nm for the three methods, respectively.

Rageh et al., [32] have proposed spectrophotometric method for the determination of cefaclor monohydrate, cefadroxil monohydrate, cephalexin anhydrous, cefradine anhydrous, cefotaxime sodium, cefoperazone sodium, ceftriaxone sodium, ceftazidime penthydrate, cefazolin sodium, cefixime and cefpodoximeproxetil, in bulk drug and in pharmaceutical formulations. The method was based on hydrolysis of the studied drugs using 0.5 M NaOH at 100°C and subsequent reaction of the formed sulfide ion with NBD-Cl (4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole) to form a yellow-colored chromogen measured at 390 nm.

Alothman and Abdalla [33] developed a spectrophotometric method for the determination of cephalexin. The method was based on the hydrolysis of the drug in sodium hydroxide solution to produce the sulphide ion and the conversion of the sulphide with the p-phenylenediamine to form a violet color product. The absorbance of the violet dye was measured at 595 nm. The method was successfully applied to the assay of cephalexin in drug formulations.

Additionally, Agbaba et al., [34] described a spectrophotometric method for determination of cephalexin, cefixime, ceftriaxone and cefotaxime, in bulk and in pharmaceuticals using the ferrihydroxamate.

Arun et al., [35] reported a spectrophotometric method in UV region for the determination of ceftazidime in bulk dosage form. The solution of ceftazidime in 0.1N HCl shows maximum absorbance at 261nm, Beer's law was obeyed in the concentration range of 2-10 µg mL^{-1}.

Moreover, De Paula et al., [36] described a spectrophotometric method for the determination of cephalexin using quinalizarin in dimethylsulfoxide medium. The limit of detection (LOD, S/N=3) and the limit of quantitation (LOQ S/N=10) were found to be 0.46 µg mL^{-1} and 1.5 µg mL^{-1}, respectively. Lakshmi and co-workers [37] reported three spectrophotometric methods (A, B and C) in the UV region for the determination of ceftriaxone sodium in vials. Method A was based on the measurement of intensity of UV radiation at 258.8 nm using 0.1 M sodium hydroxide, Method B was based on the measurement of intensity of UV radiation at 266.4 nm using 0.1 M hydrochloric acid and Method C was based on the reaction with imidazole - mercury (II) reagent in slightly acidic condition and heating at 83°C for 20 min, which showed absorbance at 236 nm. Beer's Law was obeyed in the concentration ranges 1-5, 10-60, and 0.4-2.0 µg mL^{-1} for method A, method B, and method C, respectively.

Pasha and Narayana [38] proposed spectrophotometric method for the determination of cephalexin with variamine blue. The determination is based on the hydrolysis of β-lactam ring of cephalexin with sodium hydroxide which subsequently reacts with iodate to liberate iodine in acidic medium. The liberated iodine oxidizes variamine blue to violet colored species of maximum absorption at 556 nm. The absorbance is measured within the pH range of 4.0- 4.2. Beer's law is obeyed in the range of 0.5-8.5 µg mL^{-1}. The method was applied successfully for the determination of cephalexin in pharmaceuticals.

A kinetic spectrophotometric method for the determination of cefotaxime sodium, cephapirin sodium, cephradinedihydrate, cephalexin monohydrate, ceftazidimepentahydrate, cefazoline sodium, ceftriaxone sodium, and cefuroxime sodium in commercial dosage forms has successfully been developed. The method is based on oxidation of the drug with alkaline potassium permanganate which was described by Omar et al., [39]. The reaction is followed spectrophotometrically by measuring the rate of change of absorbance at 610 nm. The calibration graph is linear in the concentration ranges of 5-15 and 5-25 µg mL^{-1} for initial rate and fixed time methods, respectively.

Nkeomaet al., [40] proposed a spectrophotometric method for determination of ceftriaxone, cefotaxime and cefuroxime in pharmaceutical dosage form. The method is based on the formation of prussian blue (PB) complex. The reaction between the acidic hydrolysis product of the antibiotics with the mixture of Fe^{3+} and hexacyanoferate (III) ions was evaluated for the spectrophotometric determination of the antibiotics. The maximum absorbance of the colored complex occurred at 700 nm. Under optimum conditions the absorbance of the PB complex was found to increase linearly with increase in concentrations of ceftriaxone, cefotaxime and cefuroxime, which corroborated with the correlation coefficient values. The calibration curves were linear in range of 2-20 and 2-18 g mL^{-1} for ceftriaxone and cefotaxim, respectively.

Pritamand and co-workers [41] reported a UV spectrophotometric method for the determination of cefuroxime axetil in bulk and in formulation. The λ_{max} of cefuroxime axetil in 0.1 N HCl was found to be 281 nm. The drug was found to be linear in the concentration range 0.4-2 µg mL^{-1} with correlation coefficient value of 0.998.

The analysis of cefixime in pure form and in pharmaceuticals through complexation with Cu^{2+} using acetate-NaOH buffer in mixture water: methanol was also conducted. The complex has maximum absorbance at λ= 410 nm. The reaction between cefixime and Cu^{2+} occurred at a stoichiometric ratio of 1:1. All reaction conditions were optimized to obtain the complex. Under optimum conditions, Beer's law was obeyed at concentrations ranging from 0.2267-22.671 µg mL^{-1} as prescribed by Ramadan et al., [42].

Ayad et al., [43] described a spectrophotometric procedure for the determination of certain cephalosporins (cefotaxime sodium and cefuroxime sodium). The method was based on the charge-transfer complex formation

between these drugs as n-donors and 7,7,8,8-tetracyanoquinodimethane (TCNQ) or p-chloranilic acid (p-CA) as pi-acceptors to give highly colored complex species. The colored products were measured spectrophotometrically at 838 and 529 nm for TCNQ and p-CA, respectively. Beer's law is obeyed in a concentration range of 7.6-15.2 and 7.1-20.0 µg mL^{-1} with TCNQ, 95.0-427.5 and 89.0-400.5 µg mL^{-1} with p-CA for cefotaxime sodium and cefuroxime sodium, respectively.

Asad et al., [44] described spectrophotometric method for the determination of cefaclor in pharmaceutical raw and dosage forms based on reaction with ninhydrin. The method was optimized and validated. The purple color (Ruhemenn's purple) that resulted from the reaction was stabilized and measured at 560 nm. Beer's law was obeyed in the concentration range of 4-80 µg mL^{-1} with molar absorptivity of 1.42×10^5 L mol^{-1} cm^{-1}.

Chavala et al., [45] described a procedure for the determination of ceftazidime and cefepime based on their condensation reaction with 1-naphthoquinolone-4-sulphonate (NQS) in alkaline media to yield orange colored products. Ceftazidime and cefepime showed maximum absorbance at 495 nm and 475 nm with linearity in the concentration range of 20-80 and 20-140 µg mL^{-1}, respectively. The proposed method was applied for the assay of ceftazidime and cefepime in commercial injection preparations. Ali et al., [46] also reported a method for the determination of some cephalosporins based on their condensation reaction with NQS.

A number of methods for analysis of cephalosporins based on their reaction with 8-hydroxy-1,3,6-pyrenetrisulfonic acid trisodium salt (HPTS) were presented by Elbashir and co-worker [47,48] and the optimum experimental reactions conditions were examined.

UV-spectrophotometric method for determination of cefadzoline sodium using purified water and phosphate buffer was proposed by Pedroso and Salgado [49]. The absorbance was measured at 270 nm and the linear range was between 8-28 µg mL^{-1}.

Determination of cefiximein in pharmaceutical formulations based on the complexation reaction between cefixime and palladium ion in the presence of acidic buffer solution (pH 3) in ethanol-distilled water medium at room temperature was proposed by Syed et al., [50]. The complex absorbed maximally at 352 nm. Beer's law was obeyed in the working concentration range of 2.5-35 µg mL^{-1}. The LOD and LOQ for the proposed method were 0.175 and 0.583 µg mL^{-1}, respectively.

Spectrophotometric method for the determination of some cephalosporins based on the hydrolysis of the cephalosporin in sodium hydroxide solution to produce the sulphide ion and the conversion of the sulphide with the p-phenylenediamine to form a violet color was reported by Alothman and Abdalla [51]. Acetaminophen was hydrolysed in sulphuric solution and the resulting p-aminophenol was oxidized with sulphide ion in the presence of Fe^{3+} to form a red product. The method was successfully applied to the assay of some cephalosporins in drug formulations.

Ni et al., [52] reported a kinetic spectrophotometric method for the simultaneous determination of three cephalosporin antibiotics, cephradine, cefaclor and cefixime, with the aid of chemometrics. The method was relied on their oxidation with potassium permangnate to produce green manganate with different kinetic rates in alkaline medium.

UV spectrophotometric method for the determination of ceftiofur sodium in the drug substance and sterile powder for injection was developed and validated by Souza et al., [53]. The UV spectrophotometric determinations were performed at 292 nm. Good linearity was obtained between 2.5-20.0 µg mL^{-1}. A prospective validation showed that the method is linear with a correlation coefficient of 0.9999.

Zhao et al., [54] proposed indirect spectrophotometric determination of sodium ceftriaxone. Sodium ceftriaxone was degraded completely in the presence of 0.20 mol L^{-1} sodium hydroxide in boiling water bath for 20 min. The method was linear in the range of 0.70-32 µg mL^{-1} and the LOD was found to be 0.60 µg mL^{-1}.

A visible spectrophotometric method for the determination of cefixime based on the formation of colored species with ferric chloride in 0.7% HCl to produce a yellowish green colored solution (λ_{max} at 430 nm) was described by Choragudi and Settaluri [55]. The method was successfully employed for the determination of cefixime in various pharmaceutical preparations.

Amin and Ragab [56] proposed spectrophotometeric method for the assay of cefotaxime sodium, cefuroxime sodium, and ceftriaxone disodium with metol-chromium (VI) reagent. The procedure was based on direct oxidation of metol by potassium dichromate in presence of drug in acidic medium and subsequent formation of ternary complex. Beer's law was obeyed in the range 0.2-28 µg mL^{-1} at λ_{max} 520 nm. The proposed method was applied to the determination of the examined drugs in pharmaceutical formulations.

Buhl and Szpikowska-Sroka [57] reported spectrophotometric method for the determination of cefotaxime, ceftriaxone and cefradine with leuco crystal violet. The determination was based on the reduction of potassium iodate in acidic medium, followed by hydrolysis of β-lactam ring of cephalosporins with sodium hydroxide. The formed iodine reacts with leuco crystal violet. The crystal violet dye of maximum absorption at 588 nm is formed. Beer's law was obeyed in the concentration range 0.8-4.8, 0.4-1.6 and 0.2-2.0 µg mL^{-1} for cefotaxime, ceftriaxone and cefradine, respectively. The molar absorptivity of the colored compound is 8.4×10^4 L mol^{-1} cm^{-1} for cefotaxime, 2.4×10^5 L mol^{-1} cm^{-1} for ceftriaxone and 1.6×10^5 L mol^{-1} cm^{-1} for cefradine. The analytical parameters were optimized and the method was successfully applied to the determination of cefotaxime, ceftriaxone and cefradine in pharmaceuticals.

Rind et al., [58] reported spectrophotometric method for the determination of the potent antibiotic ceftriaxone by derivatization with 4-dimethylaminobenzaldehyde (DAB). The derivative indicated molar absorptivity of 5.3×10^3 L mol^{-1} cm^{-1} at maximum absorption 397 nm and it obeyed the Beer's law in the range 20-100 µg mL^{-1}.

Nawal et al., [59] presented spectrophotometric method for the determination of cefepime. The method was based on the complexation of the drug with mercuric nitrate $Hg(NO_3)_2$ in acid medium (pH 3.5) and measuring the absorbance at 263 nm. Under the optimized conditions, linear relationship with good correlation coefficient (0.999)

was found between the absorbance and the concentration in the range of 3.65-40 µg mL^{-1}.

Flow-injection spectrophotometric method for the determination of cefadroxil (I) and cefotaxime (II) was described by Metwally et al., [60]. The method was based on the hydrolysis of the cephalosporin with sodium hydroxide whereby the sulfide ion was produced. Linear calibration curves were obtained in the range 36.34-109.2 and 95.48-477.4 µg mL^{-1} for I and II, respectively. The experimental LODs were 0.036 and 0.048 µg mL^{-1} for I and II, respectively. The total flow-rate is 5.3 mL min^{-1} for both drugs. Alternately, the sulfide ion produced is allowed to react with p-phenylenediaminedihydrochloride (PPDD) and Fe^{3+}, and the violet color produced is measured at 597 nm (method B). Linear calibration graphs are obtained in the range 0.5-400 and 0.5-450 µg mL^{-1} for I and II, respectively. The LODs were 0.4 and 0.2 µg mL^{-1} for I and II, respectively. The methods were successfully applied to the analysis of some pharmaceutical formulations, particularly of the injection and capsule types.

Another flow injection analysis method for the determination of cefotaxime, cefuroxime, ceftriaxone, cefaclor, cefixime, ceftizoxime, and cephalexin was proposed by Al-Momani [61]. Aliquots of each cephalosporin was hydrolyzed for 15 min with 0.1 M NaOH at 80°C and then oxidized with Fe^{3+} in sulphuric acid medium to produce Fe^{2+}. The produced Fe^{2+} is then complexed by *o*-phenanthroline (*o*-phen) in citrate buffer at pH 4.2 to form the red complex, Fe (*o*-phen)$_3^{2+}$, which exhibits maximum absorption at 510 nm. Variables such as acidity, reagent concentrations, flow rate of reagents and other parameters were also optimized.

El Walily et al., [62] proposed spectrophotometric and spectrofluorimetric procedures for the quantitative determination cephalosporins. The methods were based on the acidic oxidation of the antibiotics with Ce^{4+} at elevated temperature. Each antibiotic was determined by measuring the absorbance difference at 317 nm for spectrophotometric and by measuring fluorescence at 256 and 356 nm for excitation and emission wavelengths, respectively. The procedure has been successfully applied to the assay of these antibiotics in their pharmaceutical dosage forms. The obtained results have been statistically compared with those obtained by the official methods. The same authors [63] described fluorimetric method for the determination of cefadroxil, through the formation of its coumarin derivatives based on the reaction between the drug and ethyl acetoacetate, in acidic medium, to give yellow fluorescent products with excitation wavelength of 467 nm and emission wavelength of 503 nm.

Ródenas et al., [64] described spectrophotometric method for the determination of cephradine or ceftazidine based on the formation of compounds between these drugs and Pd^{2+}. The calibration graphs resulting from the measurement of the absorbance at 330 nm was linear over the range 5.0-60.0 µg mL^{-1} for cephradine and 3.0-60.0 µg mL^{-1} for ceftazidine.

Kumar et al., [65] proposed direct UV-spectrophotometric method for the estimation of cefixime and ofloxacin in tablet dosage forms. Two wavelengths were selected for formation and solving the simultaneous equations were 285.8 nm for cefixime and 296.6 nm for ofloxacin. The recovery studies were found close to 100% that indicates accuracy and precision of the proposed methods.

Manoj et al., [66] presented spectrophotometric procedure for the simultaneous estimation of cefotaxime sodium and sulbactam sodium in parental dosage forms. The method involved determination using the simultaneous equation method. The sampling wavelengths selected are 231.0 nm and 260.2 nm over the concentration ranges of 5-30 and 2.5-15 µg mL^{-1} for cefotaxime sodium and sulbactam sodium, respectively.

Binglin et al., [67] presented spectrophotometric assay for determination of cefotaxime based on its reaction with NQS as a chromogenic reagent. Absorbance was linear with the concentration of cefotaxime in the range of 3.8–114.6 µg mL^{-1}. The LOD and the %RSD are 3 mg L^{-1} and 1.2%, respectively. The average recovery is in the range of 98.9–101.2%. The results indicate that the method could be applied to the determination of cefotaxime in injections.

Mouyed and co-workers [68] developed a batch and flow injection spectrophotometric methods for analysis of cefotaxime sodium in pure form and injection these methods were based on a diazotization and coupling reaction between diazotized cefotaxime sodium and thymol in alkaline medium to form an intense, stable, red and water soluble dye which has a maximum absorption at 522 nm. Beer's law was obeyed over the concentration range 8-160 and 50-500 µg mL^{-1} with LODs of 1.56 and 1.32 µg mL^{-1} for the batch and flow injection methods, respectively.

Thabit et al., [69] proposed a spectrophotometric method for the determination of cephradine and cefadroxil in their pure forms as well as in some pharmaceutical dosage forms. The method is based on the formation of a charge-transfer complex between 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ) as π-acceptor and the studied drugs as n-donors in aqueous medium in the presenceof borate buffer solution of pH 9. The spectra of the complexes show absorption maxima at 342 and 338 nm with the apparent molar absorptivities of 9.61×10^3 and 1.52×10^4 L mol^{-1} cm^{-1} for cephradine and cefadroxil, respectively. Beer's law was obeyed in the concentration ranges of 1.2-17 and 1.6-20 µg mL^{-1}, respectively.

Spectrophotometric method for the determination of cefotaxime was developed and validated by Kumar et al., [70]. The method was based on the condensation of cefotaxime with NQS in alkaline media to yield orange colored products. Cefotaxime showed maximum absorbance at 475 nm with linearity which was observed in the concentration range of 20-140 µg mL^{-1}.

Adegokeand Quadri [71] reported a spectrophotometric method for the analysis of some cephalosporins namely; ceftriaxone, ceftazidime, cefixime, cefotaxime and cefuroxime in bulk samples and pharmaceutical dosage forms. The reaction involves a two-step process of diazotization of the cephalosporins with acidified $NaNO_2$ at temperature range 0-5°C and coupling with acidified p-dimethylaminobenzaldehyde (DMAB). The reaction with DMAB occurred at a stoichiometric ratio of 1:1. Optimization of DMAB concentration revealed the superiority of using 0.3% DMAB in 0.0625 M H_2SO_4 with the best diluting solvent being methanol.

Spectrophotometric method for the determination of cephalosporins namely; cefaclor, cefazolin, cefotaxime, cefoxitin and cefamandole nafate in pharmaceuticals and

bulk was described by Issopoulos [72,73]. The method was based on the reaction with $(NH_4)_6Mo_7O_{24}$ and 0.5 M sulfuric acid and measured the absorbance of the blue colored solution at 810 nm. A linear relation between the absorbance and anion was observed for all antibiotics studied. The recoveries and relative standard deviations were 96.7-104.7 and 0.60-2.08 %, respectively. The same author used molybdo phosphoric acid as an oxidizing agent for the spectrophotometric determination of 4 cephalosporin derivatives; cefadroxil (I), cefapirin (II), ceforanide L-lysine (III) and cefuroxime (IV) in pure form or in pharmaceutical formulations. Beer's law was obeyed up to 100 µg mL^{-1} for I, up to 60 µg mL^{-1} for II and IV and up to 80 µg mL^{-1} for III. The molar absorptivities were 4.58×10^3, 11.3×10^3, 9.8×10^3 and 10.9×10^3 L $mol^{-1}cm^{-1}$ and the sandell sensitivities were 83.3, 39.3, 53.0 and 41.0 ng cm^{-2} for I, II, III and IV, respectively.

Alaa and Ragab [74] described spectrophotometric determination of certain cephalosporins in pure form and in pharmaceutical formulations with metolchromium (VI) reagent. Beer's law was obeyed in the range 0.2-28 µg mL^{-1} at maximum absorption 520 nm.

Spectrophotometric determination of some cephalosporins using palladium (II) chloride was developed El-Ansary et al., [75]. The method was based on the reaction of cephalosporins with palladium (II) chloride in the pH range (2.5-6.0) and yellow water-soluble complexes formed with maximum absorbance at 337-350 nm. Beer's law was obeyed in the concentration range of 1.5-12.6, 2.0-14.4 and 3.0-19.2 µg mL^{-1} of cefadroxil, cephradine and cefotaxime, respectively. The proposed method was used for the determination of the above mentioned drugs in their pharmaceutical preparations.

Determination of cefadroxilin pharmaceutical formulations pharmaceutical formulations using Lawsonia inermis (Henna) as natural reagent was presented by Hassan et al., [76]. The proposed method was based on the formation of yellow colored product (λ_{max} = 410 nm) that resulted after addition of the reagent, which is composed of a mixture of Henna extract, sodium hydroxide and potassium permanganate, to cefadroxil aqueous solution. Beer's law is obeyed over the concentrations of 35-100 µg mL^{-1}, with a linear regression correlation coefficient of 0.996.

A spectrophotometric method was described for the assay of cefadroxil in tablets by Mallapu et al., [77]. The cefadroxil was brominated with bromate – bromide mixture under strong acidic conditions. After bromination the excess brominating mixture was treated with methylene blue resulting in the formation of stable grass green colored complex. The absorbance of the grass green color was measured at 670 nm against the blank solution. Beer's law is obeyed over the concentrations of 100-800 µg mL^{-1}. The proposed method is successfully applied to the determination of cefadroxil in tablets. Reported spectrophotometric for determination of cephalosporins in pharmaceutical formulation are summarized in Table 3.

Table 3. Reported spectrophotometric methods for the determination of cephalosporins

Reagents for Complexation	λ_{max} (nm)	Linear Range	Derivatized Drug	Applications	Reference
Ascorbic acid	410	3-15	Ampicillin, amoxicillin, cephalexin, cefadroxil and cefaclor	Pharmaceutical preparations	24
σ-acceptor iodine, the π-acceptors: 2,3-dichloro-5,6-dicyano-*p*-benzo-quinone (DDQ)	520	20-320	Cephapirin sodium, cefazoline sodium, cephalexin monohydrate, cefadroxil monohydrate, cefotaxime sodium, cefoperazone sodium and ceftazidime pentahydrate	Pure form & pharmaceutical formulations	12
ρ-chloranilic acid (ρ-CA)	520	2.54-42.83	-	Pure form & pharmaceutical formulations	25
2,3,5-triphenyltetrazolium chloride (TTC) blue tetrazolium [3,3′-(3,3′-dimethoxy-4,4′-biphenylene) bis(2,5-diphenyl-2H-tetrazolium chloride] (BT) quercetin, N-bromosuccinimide N-chlorosuccinimide	485 525	2-24 2-24	Cefaclor monohydrate, cefotaxime sodium, ceftazidime pentahydrate, ceftriaxone sodium, cephalexin monohydrate, and cephradine	Pure form & pharmaceutical formulations	26
Hydrochloric acid(method1) Sodium hydroxide(method2)	281 (method1) 300 (method 2)	2-30 4-30	Cefuroxime axetil	Bulk drug and tablet	27
Sodium hydroxide+iodine & wool fast	540	0.8-9.6	Cefadroxil, ceftezoxime	Drug	28
Ferric chloride and potassium ferricyanide	791	1-6	Cephalexin	Capsules	29
Folin-ciocalteu	752 750	5-60 2-36	Cefotaxime Ceftriaxone	Pharmaceutical formulations	30
Ammonium molybdate and sulphuric acid (AM/H_2SO_4) (1st method) ferric chloride/ ortho phenonthraline (2nd method) Ferric chloride /potassium Ferricyanide solutions (3rd method)	625 510 740	10-60 1.25-7.5 2.5-15	7-Amino deacetoxy cephalosporanic acid (7-ADCA)	Pharmaceutical formulations and bulk	31
(4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole	390	5-160	Cefaclor monohydrate, cefadroxil monohydrate, Cefalexin anhydrous, cefradine anhydrous,	Bulk drug and pharmaceutical formulations	32

			cefotaxime Sodium, cefoperazone sodium, ceftriaxone Sodium, ceftazidime penthydrate, cefazolin Sodium, cefixime and cefpodoxime proxetil		
Sodium hydroxide and p-phenylenediamine	595	0.1-50	Cephalexin	Drug formulations	33
Ferrihydroxamate	-	-	Cephalexin, cefixime, ceftriaxone and cefotaxime	Pure form & pharmaceutical formulations	34
Hydrochloric acid	261	2-10	Ceftazidime	Pure form & pharmaceutical formulations	35
Quinalizarin in dimethylsulfoxide	-	-	Cephalexin	Commercial pharmaceutical formulations	36
Sodium hydroxide Hyochloric acid Imidazole - mercury (II)	258.8 266.4 236	1-5 10-60 0.4-2	Ceftriaxone sodium	Vials	37
Variamine blue	556	0.5- 8.5	Cephalexin	Drug formulations	38
Potassium permanganate degradation	610	5-15 (initial rate) 5-25 (fixed time)	Cefotaxime sodium, Cephapirin sodium, Cephradine dihydrate, Cephalexin monohydrate, Ceftazidime pentahydrate, Cefazoline sodium, Ceftriaxone sodium, and Cefuroxime sodium	Commercial dosage forms	39
Cerous (IV) sulfate	-	-	Cephalexin	Pharmaceutical preparations	40
Alkali-induced degradation products	265-230	-	Cefotaxime, ceftriaxone and ceftazidime	Injection	41
Degradation products	268.6, 306 228.6	-	Ceftazidime, Cefuroxime sodium, cefotaxime sodium	Pharmaceutical preparations	42
7,7,8,8-tetracyanoquinodimethane (TCNQ) p-chloranilic acid (p-CA)	838 529	7.6-15.2 and 7.1-20.0 95.0-427.5 and 89.0-400.5	Cefotaxime sodium Cefuroxime sodium	Pharmaceutical preparations	43
Ninhydrin	560	4-80	Cefaclor	Pharmaceutical preparations	44
1-naphthoquinolone 4 sulphonate (NQS)	495 475	20-80 0.3-3 20-140	Ceftazidime Cefepime	Commercial injection preparations	45
1-naphthoquinolone 4 sulphonate (NQS)	521 455 493	0.5-2.8 0.8-2.8 0.2-1.2	Cefixime Cephalexin Cefotaxime sodium	Pharmaceutical preparations	46
1-hydroxy-3, 6, 8-pyrenetrisulfonic acid trisodium salt, (HPTS)	470 480 479	0.1-0.6 0.3-1.8 0.5-3	Cefixime Cephalexin Cefotaxime sodium	Pharmaceutical preparations	47
1-hydroxy-3, 6, 8-pyrenetrisulfonic acid trisodium salt, (HPTS)	485 475	1-6 0.5-3	Cefadroxile Cefuroxime sodium	Pharmaceutical preparations	48
Purified water + phosphate buffer	270	8-28	Cefadzoline sodium	Powder for injection	49
Palladium ion	352	2.5-35	Cefixime	Pharmaceutical preparations	50
p-phenylenediamine	505	1-50	Cephalexin, Cephradine, Cephalothin, Cephaloridine and Cephoxazole	Capsules and tablets	51
Potassium permangnate	-	-	Cephradine, cefaclor and cefixime	Pure form and in pharmaceutical formulations	52
	292	2.5-20	Ceftiofur sodium	Drug and sterile powder	53
Sodium hydroxide	-	0.7-32	Sodium ceftriaxone	Pharmaceutical preparations	54
Hydrochloric acid	430	-	Cefixime	Pharmaceutical preparations	55
Metol-chromium(VI)	520 525	0.2-28	Cefotaxime sodium, cefuroxime sodium, and ceftriaxone disodium	Pharmaceutical preparations	56
Leuco crystal violet	588	0.8-4.8 0.4-1.6 0.2-2.0	Cefotaxime Ceftriaxone Cefradine	Pharmaceutical preparations	57
4-dimethylaminobenzaldehyde	397	20-100	Ceftriaxone	Pharmaceutical preparations	58
Mercuric nitrate	-	-	Cefepime	Pure form and in pharmaceutical formulations	59
Sodium hydroxide	670	36.34-109.2	Cefadroxil	Injection and	60

		95.48-477.4	Cefotaxime	capsules	
Sodium hydroxide	510	7.6-15.2 7.1-20.0	Cefotaxime, cefuroxime, ceftriaxone, cefaclor, cefixime, ceftizoxime, and cephalexin	Pharmaceutical formulations	61
Cerium (IV)	-	317	Cefadroxil, Cefamandole nafate, Cefuroxime axetil or sodium, Cefaclor, Ceftazidime, Ceftizoxime, Ceftriaxone, Cefoperazone, Cefixime and Cefpodoxime proxetil	Pharmaceutical dosage forms	62
Pd(II)	330	5.0-60 3.0-60	Cephradine Ceftazidine	Pharmaceutical dosage form	63
Methanol + hydrochloric acid	285.5	5-40	Cefixime	Pharmaceutical formulations	64
	231.0 and 260.2	5-30 2.5-15	Cefotaxime Sodium Sulbactam Sodium	Parentral dosage form	65
Sodium 1,2-naphthoquinone-4-sulfonate (NQS)	489	3.8-114.6	Cefixime	Injection	66
Thymol	522	8-160 (batch method) 50-500 (flow injection method)	Cefotaxime sodium	Pure form and injection	67
1,2-naphthoquinone-4-sulfonate (NQS)	489	3.8-114.6	Cefotaxime		68
2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)	342 338	1.2-17 1.6-20	Cephradine Cefadroxil	Pure and pharmaceutical forms	69
1,2-naphthoquinone-4-sulfonate (NQS)	475	20-140	Cefotaxime sodium	Bulk and pharmaceutical dosage form	70
Acidified *p*-dimethylaminobenzaldehyde	420 420 430 400 420	25-60 5-35 30-55 20-45 10-40	Ceftriaxone Ceftazidime Cefixime Cefotaxime Cefuroxime	Bulk sample and pharmaceutical dosage form	71
$(NH_4)_6Mo_7O_{24}+H_2SO_4$	810	20-120 20-140	Cefaclor, cefazolin, cefotaxime, cefoxitin cefamandole nafate	Pharmaceutical forms	72
Molybdophosphoric acid	-	Up to 100 Up to 60 Up to 80 Up to 60	Cefadroxil Cefapirin Ceforanide L-lysine Cefuroxime	Pharmaceutical forms	73
Metol-chromium	520	0.2-28	Cefotaxime sodium Cefuroxime sodium Ceftriaxone disodium	Pharmaceutical forms	74
Palladium(II) chloride	373-350	1.5-12.6 2.0-14.4 3.0-19.2	Cefadroxil Cephradine Cefotaxime	Pharmaceutical forms	75
Lawsonia inermis (Henna)	410	35-100	Cefadroxil	Pharmaceutical forms	76
Bromate - bromide	670	100-800	Cefadroxil	Tablet	77

2.2. Spectrofluorometric Methods

Fluorimetric method for the determination of cefadroxil and cephalexin based on the reaction of the drugs with fluorescamine at a specific pH, ranging from 7.8-8.4, was described by Siddaiah et al., [78]. The produced derivatives exhibit maximum fluorescence intensities at 472 nm after excitation at 370 nm. The method was applied to the determination of these drugs in pharmaceutical formulations.

Omar et al., [79] developed kinetic spectrofluorimetric method for the determination of cephalexin, cefotaxime and cefuroxime. The method was based on their degradation under an alkaline condition producing fluorescent products. The reaction was followed spectrofluorimetrically by measuring the rate of change of fluorescence intensity at specified emission wavelength. The initial rate and fixed time methods were used for the construction of calibration graphs to determine the concentration of the studied drugs. The calibration graphs were linear in the concentration ranges 0.2-1.2 and 0.2-2.2 µg mL^{-1} using the initial rate and fixed time methods, respectively.

Bukhari et al., [80] investigated spectrofluorimetric method for the assay of cefixime using calcein fluorescence probe. Under the optimum conditions, the fluorescence intensity was linear with the concentration of cefixime in the range of 1.33×10^{-7}-1.60×10^{-4} mol L^{-1}. The proposed method was applied for the quantification of cefixime in pharmaceutical formulations.

Spectrofluorimetric determination of cefixime in pure form and pharmaceutical preparations based on reaction with 2-cyanoacetamide in the presence of 21% ammonia at 100℃ was developed Jasmin et al., [81]. The fluorescent reaction product showed maximum fluorescence intensity at 378 nm after excitation at 330 nm. The fluorescence intensity versus concentration plot was linear over the range of 0.02-4 µg mL^{-1}. Yang et al., [82] developed spectrofluorimetric method for determination of cefadroxil in pharmaceutical formulations after mixing the drug with fluorescein-Hg and 1.0 mol L^{-1} sodium hydroxide.

Elbashir and co-workers [83,84,85] proposed spectrofluorometric methods for analysis cephalosporins, in pharmaceutical formulations. The methods were based on a reaction between cephalosporins with NQS in alkaline medium, to give highly fluorescent derivatives extracted with chloroform and subsequently measured with spectrofluorimetry. The method is based on a reaction between cephalosporins namely; cefixime, cephalexine, and cefotaxime sodium with HPTS in alkaline medium, at pH 12.0 was also developed and validated. Beer's law is obeyed over concentrations of 10-60, 5-35 and 10-60 ng mL^{-1} for cefixime, cephalexine, and cefotaxime sodium, respectively.

Manzoori et al., [86] described fluorimetric method for determination of cefixime based on fluorescence quenching of terbium danofloxacin (Tb^{3+}-Dano) in the presence of cefixime with maximum excitation and emission wavelengths at 347 nm and 545 nm, respectively. The quenched fluorescence intensity of Tb^{3+} - Dano system is proportional to the concentration of cefixime. The optimum conditions for the determination of cefixime were studied, the developed method was evaluated in terms of accuracy, precision and limit of detection. The linear concentration ranges for quantification of cefixime were 8.8×10^{-8}- 8.8×10^{-7} mol L^{-1}. The cefixime was determined in pharmaceutical tablets. Reported spectrofluorometric methods for the determination of cephalosporins in pharmaceutical formulation are summarized in Table 4.

Table 4. Reported spectrofluorimetric methods for the determination of cephalosporins

Reagents for Complexation	λ_{ex}/ λ_{em}(nm)	Linear Range	Derivatized Drug	Applications	Reference
Coumarin	476/503	-	Cefadroxil	Pharmaceutical formulations	63
Fluorescamine	370/472	5-30 ng mL^{-1}	Cefadroxil Cephalexin	Pharmaceutical formulations	78
Drug in alkaline medium	-	0.2-1.2 µg mL^{-1} (Initial rate) 0.2-2.2 µg mL^{-1} (Fixed time)	Cephalexin Cefotaxime Cefuroxime	Pharmaceutical formulations	79
Calcein (CA)	-	1.33×10^{-7}-1.60×10^{-4} mol L^{-1}	Cefixime	Pharmaceutical formulations	80
2-cyanoacetamide	330/378	0.02-4 µg mL^{-1}	Cefixime	Pure form and pharmaceutical preparations	81
Fluorescein-Hg	-	-	Cefadroxil	Pharmaceutical formulations	82
1,2-naphthoquinone-4-sulfonic (NQS)	520/600 455/580 490/580	10-35 ng mL^{-1} 10-60 ng mL^{-1} 20-45 ng mL^{-1}	Cefixime Cephalexine Cefotaxime sodium	Pharmaceutical formulations	83
1,2-naphthoquinone-4-sulfonic (NQS)	470/610 460/605	20-70 ng mL^{-1} 15-40 ng mL^{-1}	Cefadroxile Cefuroxime sodium	Pharmaceutical formulations	84
8-hydroxy-1,3,6 pyrenetrisulfonic acid trisodium salt (HPTS)	480/520 470/500 480/510	10-60 ng mL^{-1} 5-35 ng mL^{-1} 10-60 ng mL^{-1}	Cefixime Cephalexine Cefotaxime sodium	Pharmaceutical formulations	85
Terbium-danofloxacin (Tb^{3+}-Dano)	347/545	8.8×10^{-8}-8.8×10^{-7} mol L^{-1}	Cefixime	Tablet	86

3. Conclusions

In this review analysis of cephalosporins by spectroscopic methods is presented. Most of the reported methods are visible spectrophotometric methods. These methods are based on the formation of colored product due to reaction of the cephalosporins with charge transfer and oxidizing agent reagents, or ion pair formation. The methods have been used for the quantitative determination of the drug in pure form and commercial preparations. The commonly occurring excipients do not interfere in the determination of the drug in the case of commercial samples.

Statement of Competing Interests

The authors have no competing interests.

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