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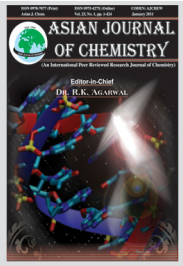

REVIEW

Analysis of Two Cephalosporin Drugs Ceftriaxone and Cefepime Alone and in Presence of Two β-Lactamase Inhibitors Sulbactam and Tazobactam

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The β-lactam is the most widely prescribed antibiotic of the all antibiotics. The most important problem related with this type of antibiotic is their susceptibility to β-lactamases which renders them ineffective. Therefore, use of inhibitors of β-lactamases that are structurally similar to β-lactam antibiotics are very much essential. In this review, the authors have extensively presented the analytical methods available for two widely used cephalosporin drugs ceftriaxone and cefepime along with two β-lactamases inhibitors sulbactam and tazobactam.

Key Words: Ceftriaxone, Cefepime, Sulbactam, Tazobactam, β-lactamase inhibitors.

INTRODUCTION

The β-lactam family is the biggest and most important class of clinical antibiotics[1]. Not only the biggest, it is also the safest and the most effective broad spectrum bactericidal antimicrobial agents available to the clinician and have therefore become the most widely prescribed of all antibiotics[2]. Their sales are estimated at US$ 15 billion, so they represent the major biotechnology products with worldwide dosage sales at around 65 % of the total market of antibiotics[3]. All of these semi-synthetic antibiotics derive from the 7-aminocephalosporanic acid composed of a β-lactam ring fused with a dihydrothiazine ring, but differ in the nature of the substituents attached at the 3- and or 7-positions of the cephem ring. These substituent affect either the pharmacokinetic properties (3-position) or the antibacterial spectrum (7-position) of the cephalosporins[4]. Currently, bacteria have shown resistance to β-lactam antibiotics by producing β-lactamases, which degrade and make them inactive. Despite this problem, these antibiotics are still important and effective in antimicrobial therapy in many situations, leading the pharmaceutical industry to develop strategies to contain the expansion of β-lactamases. One of the strategies to circumvent this problem is the use of inhibitors of β-lactamases that are structurally similar to β-lactam antibiotics such as clavulanic acid, sulbactam and tazobactam[5].These structurally similar β-lactamases inhibitors prevent the hydrolysis of the β-lactam ring of the penicillins and cephalosporins[6]. These agents are irreversible inhibitors of β-lactamase enzyme and possess low antibacterial activity. They irreversibly inhibit many plasmid mediated and some chromosomal β-lactamases. They increases the antibacterial spectrum and clinical effectiveness of all the penicillins and cephalosporins[7]. Despite the relatively extended knowledge on these drugs, their qualitative and quantitative analysis gives rise to many problems. These difficulties are due to the chemical instability of the common β-lactam nucleus and the minor differences in chemical structures between the analogues[8]. As there is wide increase in the use of the formulations containing penicillins or cephalosporins in combination with different β-lactamases inhibitors, the use of the analytical methods to analyze them also increases. In the process of developing such simultaneous analytical methods, for this difficult class of compound a through extensive knowledge of the previously published analytical data are required. The authors here present an extensive review of the analytical methods of four different drugs ceftriaxone, cefepime, tazobactam and sulbactam either alone or in combination with any other drugs.

Ceftriaxone: Ceftriaxone sodium is chemically known as, (Z)-7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetyl amido]-3-[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-1,2,4-triazin-3-yl)thiamethyl]-3-cephem-4-carboxylic acid, disodium salt[9,10]. Ceftriaxone contains a highly acidic, heterocyclic system on the 3-thiamethyl group. This unusual dioxotriazine ring

system is believed to confer the unique pharmacokinetic properties of this agent.

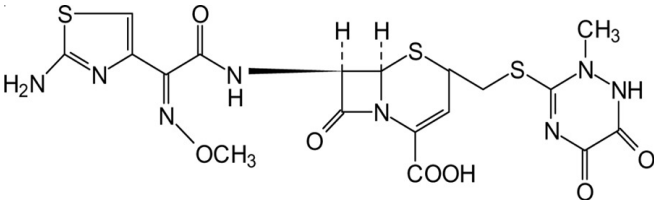

Fig. 1. Chemical structure of Ceftriaxone sodium

Ceftriaxone is almost white to yellowish-orange, slightly hygroscopic, crystalline powder having melting point of 155 ºC. It is freely soluble in water, very slightly soluble in ethanol and sparingly soluble in methanol[10,11]. In contrast to other cephalosporins, ceftriaxone possesses a greatly extended elimination half-life of (6-8 h), which has resulted in a recommended once daily administration schedule.

Analysis in bulk drug and dosage forms

Spectroscopic methods: A spectrophotometric method for the determination of ceftriaxone by derivatization with 4-dimethylaminobenzaldehyde is described. Ceftriaxone reacts with 4 dimethylaminobenzaldehyde to form an azomethine derivative 7-(2-{2-[(4-Dimethylamino-benzylidene)-amino]-thiazol-4-yl}-2-methoxyiminocetylamino)-3-(6-hydroxy-2-methyll-5-oxo-2,5-dihydro-[1,2,4]triazin-3-ylsulfanylmethyl)-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid[12]. Ceftriaxone has been analyzed using folin-ciocalteu reagent in presence of 20 % sodium carbonate solution along with cefotaxime simultaneously. The blue colour chromogen formed is measured at wavelength of maximum absorption at 750 nm against reagent blank[13]. Ceftriaxone was analyzed after reacting with imidazole- mercury (II) reagent. Imidazole catalyzes β-lactam ring and forms an oxazoline ring in the side chain. Binding of mercury (II) to the third position, which is freed by the cleavage of dihydrothiazine ring, stabilizes the product which is measured at 236 nm[14].

Chromatographic methods: RP-HPLC estimation of ceftriaxone sodium was achieved on a waters XTerra 5 µm, 250 × 0.46 mm *i.d.*, RP-18, column using photodiode array detector at 240 nm. The mobile phase consisted of 0.1 M triethylammonium acetate-acetonitrile(60:40v/v)[15]. Simultaneous estimation of ceftriaxone and tazobactam using stability indicating HPLC method was reported. The separation was obtained using a mobile phase containing a mixture of 3:2 ratio of 0.02 M phosphate buffer and 0.4 % w/v solution of tetra heptyl ammonium bromide solution in acetonitrile, with final pH of 6.6-6.8 on an octa-decyl-silane (ODS) column (4.6 × 250 mm, 5µ) with UV detection at 230 nm[16]. Chromatographic separation of ceftriaxone was achieved on a reversed-phase C_{18} microbore column (Hypersil 5 µm, 200 × 2.1 mm) with UV detection at 270 nm. The mobile phase consisted of methanol, acetonitrile and phosphate buffer in the ratio of 20:20:60, v/v/v along with cetyltrimethylammonium bromide (0.01 M) as an ion pairing agent[17]. An isocratic liquid chromatographic method with UV detection at 220 nm is described for simultaneous determination of ceftriaxone sodium and sulbactam sodium in pharmaceutical formulations. Chromatographic separation was achieved on a hypersil ODS C_{18} column using a mobile phase consisting of a binary mixture of acetonitrile and tetrabutyl ammonium hydroxide adjusted to pH 7.0 with orthophosphoric acid in ratio 70:30[18]. Separation of ceftriaxone in bulk drug and pharmaceutical formulations was achieved using Kromasil C_{18}, 250 × 4.6 mm, 5 µm column with mobile phase A containing potassium dihydrogen phosphate buffer,citric acid buffer and acetonitrile and mobile phase B containing tetradecyl ammonium bromide, tetraheptyl ammonium bromide and acetonitrile at different time intervals as eluent at a flow rate 0.8 mL/min. The detection was performed at 230 nm[19]. Simultaneous estimation of ceftriaxone and sulbactam in parenteral preparation was carried out by employing a mobile phase of phosphate buffer and acetonitrile in the ratio of 35:65. In this method ibuprofen was used as the internal standard for better accurary and reproducibility. The column used was Kromasil C_8, 150 × 4.6 mm, 5µ particle size and the detection wavelength of the column affluent was 215 nm[20]. Ceftriaxone sodium was analyzed simultaneously along with sulbactam sodium in bulk and pharmaceutical formulation by reverse phase high performance liquid chromatographic method. The separation was done by a Kromasil C_{18} column (250 mm × 4.6 mm, 5 µm) using Acetonitrile: buffer pH 7: distilled water (400:50:550, v/v/v) as mobile phase[21].

Miscellaneous techniques: Ceftriaxone is not compatible with calcium containing products so its incompatibility was evaluated using the ionic product of precipitation and the measurement of insoluble microparticles using a light obscuration particle counter. The number of insoluble microparticles with a diameter less than 10 µm in the mixed sample solution, determined using a light obscuration particle counter was increased when the ceftriaxone concentration was = 0.8 mg/mL[22].

Analysis in biological fluids

Chromatographic methods: Chromatographic separation of ceftriaxone was achieved on a reversed-phase C_{18} microbore column (hypersil 5 mm, 200 × 2.1 mm) with UV detection at 270 nm. The mobile phase consisted of methanol, acetonitrile and phosphate buffer in the ratio of 30:40:30, v/v/v along with cetyltrimethylammonium bromide (0.01 M) as an ion pairing agent. Lidocaine was used as an internal standard[17]. Ion-exchange HPLC was used for fast monitoring of the cephalosporin antibiotic ceftriaxone in blood and tissue samples. The experiments were performed on a protein chromatography system of the FPLC type equipped with a 8 × 75 mm column filled with a TSDK DEAE-5 PW sorbent(anion exchanger). The samples were eluted in a gradient mode between sodium acetate solution and a mixture of sodium acetate solution potassium chloride and acetonitrile[23]. An ion pair high-performance liquid chromatographic assay to measure ceftriaxone in serum, urine and cerebrospinal fluid was being described. The mobile phase was a combination of acetonitrile and water in the ratio of 46:54, adjusted to pH 9.0 with K_2HPO_4, which contained hexadecyltrimethylammonium bromide as the ion-pairing agent. Separation was performed at ambient temperature on a µbondapak C_{18} reverse-phase column of dimension 30 × 0.4 cm[24]. Serum concentration of ceftriaxone

was analyzed at 267 nm, along with other cephalosporins using on-line solid phase sample clean up procedure. The extraction column used was 50 × 2.1 mm I.D. dry filled with an NH_2 40-μm silica and the analytical column was a Supelcosil LC-18(150 × 4.6 mm) with the particle size of 3 μm. Wash solution consisted of a mixture of 5 % methanol in phosphate buffer and analytical mobile phase composed of methanol, acetonitrile and phosphate buffer in the ratio of 20:15:65 respectively[25]. Ceftriaxone and ceftazidime concentrations in plasma and brain dialysates were determined by high-pressure liquid chromatography with UV detection at 270 nm. The plasma samples were subjected to an extractive procedure prior to injection into the chromatograph. The mobile phase consisted of phosphate buffer, tetradecyltrimethyl ammonium bromide, acetonitrile and methanol in the ratio of 45:15:30:10; v/v for ceftriaxone analysis[26]. After a single step liquid-liquid extraction using toxi-tube A, systematic toxicological analysis of ceftriaxone was performed in human blood and urine at the detection wavelength of 245 nm. The mobile phase of the system, phosphate buffer and acetonitrile was delivered in the multi step gradient mode. Symmetry C_8, 250 × 4.6 mm I.D, 5 μm particle size column was used for the separation at 30 ºC temperature[27]. HPLC analysis of ceftriaxone was performed in plasma to study the pharmacokinetics of ceftriaxone *in vivo*. A Hypersil ODS stainless-steel column of 100 mm long and 3 mm internal diameter and 5 μm particle size was used. The mobile phase consist of 0.7 % acetonitrile in a 0.005-mol/liter acetate buffer delivered at a flow rate of 1 mL/min and the detector wavelength was set at 274nm[28]. Pharmacokinetics of ceftriaxone was studied in calves with HPLC technique. Chromatographic separation was done using Lichrocart RP-18 column (250 × 4 mm) at room temperature. The mobile phase consisted of a mixture of di-sodium hydrogen phosphate dihydrate buffer along with N-acetyl -N, N, N-trimethyl ammonium bromide and acetonitrile in the ratio of 62:38 v/v. The elute was monitored at a wavelength of 254 nm[29]. *In vivo* microdialysis was used to determine unbound ceftriaxone in rat blood. Isocratic elution of ceftriaxone within 10 min was achieved using a microbore liquid chromatographic system. The chromatographic mobile phase consisted of methanol and 100 mM monosodium phosphoric acid in the ratio of 15:85 v/v and the C_{18} microbore column used was having the dimension of, 150 ×1 mm, 5 μm, maintained at room temperature. The wavelength of the UV detector was set at 280 nm[30].

Cefepime: Cefepime is chemically (6R,7R,Z)-7-(2-(2-aminothiazol-4-yl)-2 (methoxyimino)acetamido)-3-((1-methylpyrrolidinium-1-yl)methyl)-8-oxo-5-thia1-azabicycle [4.2.0] oct-2-ene-2 carboxylate[9,10].

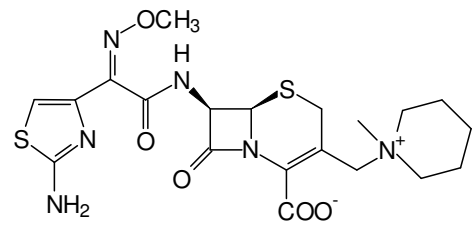

Fig. 2. Chemical structure of cefepime

It is a semisynthetic 4th generation cephalosporin. It is colourless powder having the melting point of 150 ºC[10]. Cefepime is a 4th generation cephalosporin having the elimination half-life of 2 h.

Analysis in bulk drug and dosage forms

Chromatographic methods: Analysis of cefepime along with sulbactam in pharmacetutical formulation was reported where a mobile phase of binary mixture of acetonitrile and tetrabutylammonium hydroxide was used at pH 5. Chromatographic separation was performed on a hypersil ODS C-18 column (250 × 4.6, 5 μ) and the detection wavelength used was 230 nm. The same method was applied for the analysis of plasma samples as well[31].

Analysis in biological fluids: Analysis of cefepime along with vancomycin and imipenem in human plasma of burn patients by HPLC was done using Supelcosil LC-18 column of dimension 25 cm × 4.6 mm × 5 μm. The mobile phase consisted of a mixture of 0.075 M acetate buffer of pH 5.0 and acetonitrile in the ratio of 92:8 v/v and the affluent was monitored at 230 nm[32]. Simultaneous determination of cefepime along with garenoxacin, moxifloxacin and levofloxacin in human urine by HPLC with UV detection at 256 nm for cefepime was reported. The mobile phase used was composed of acetonitrile, phosphoric acid/sodium hydroxide and *n*-octylamine in gradient mode. LiChrospher 100 RP-18 column of dimension 250 mm × 4 mm × 5 μm was used along with a pre-column[33]. Cefepime was analyzed along with cefazolin in human plasma and dialysate by using a mobile phase of dibasic potassium hydrogen phosphate and methanol in gradient mode with the detector set at 254 nm. The analytical column used was an onyx monolithic C_{18} (20cm × 4.6 mm I.D.) coupled to a C_{18} guard column[34]. Using a validated method cefepime was analyzed in several biological matrices such as serum, cerebrospinal fluid and urine and its pharmacokinetic profiles were checked. The method used a mobile phase containing phosphate buffer and methanol in the ratio of 3:1. A LiChrospher 100 RP-18 column of dimension 250 mm × 4 mm × 5 μm was used and the column affluent was monitored at 256 nm[35]. A simple and sensitive HPLC method was developed for analysis of cefepime in human plasma using an Atlantis dC 18 column (250 mm × 4.6 mm, 5 μm) and the column was eluted with methanol and dihydrogen phosphate in the ratio of 15:85 v/v as the mobile phase. The detection wavelength was set at 270 nm and the temperature of the column was maintained at 40 ºC[36]. In human serum cefepime was analyzed using Ultrasphere XL-ODS column (75 × 4.6) and a mobile phase containing acetonitrile and ammonium acetate. The flow rate was maintained at 1 mL/min and the effluent was monitored at 254 nm[37]. An HPLC assay method for the determination of cefepime and cefpirome in human serum without changing chromatographic conditions was described. The mobile phase of the method was consisted of a mixture of acetonitrile and potassium dihydrogenphosphate buffer in the ratio of 6:94 v/v with Supelcosil ABZ+ (150 × 4.6 mm, 5 μm) column. UV absorbance at a wavelength of 263 nm was used for detection[38]. A reversed-phase HPLC method was described to determine cefepime levels in plasma and vitreous fluid. Cefepime and the internal standard cefadroxil were separated

on a Shandon Hypersil BDS C_{18} column by using a mobile phase of sodium dihydrogen phosphate monohydrate and methanol in the ratio of 87:13 v/v. Ultraviolet detection was carried out at 270 nm[39]. A column-switching high-performance liquid chromatographic technique with UV detection at 280 nm was employed for the analysis of cefepime in human plasma and urine and in dialysis fluid. The HPLC column, nucleosil C_{18} (100 mm × 4.6 mm, 5 μm) was used with an eluent mixture composed of acetonitrile and ammonium acetate[40].

Tazobactam: Chemically it is (2S,3S,5R)- (9CI);4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,3-methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-ylmethyl)-4,4-dioxide[10]. It is a derivative of the penicillin nucleus and is a penicillanic acid sulfone.

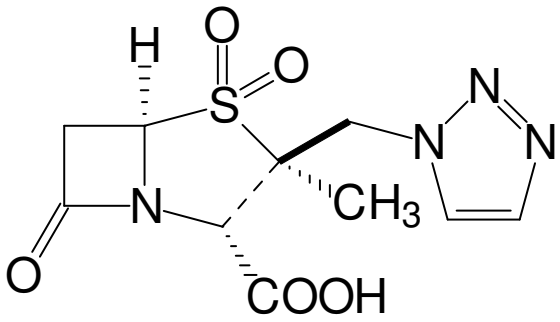

Fig. 3. Chemical structure of Tazobactam

It is an amorphous solid with melting point between 140-147 ºC. Tazobactam sodium is mainly used as lactamase inhibitor and it also used with lactam antibiotics to enhance their effect. It broadens the spectrum of penicillins and cephalosporins by making it effective against organisms that express β-lactamase and would normally degrade penicillins and cephalosporins.

Analysis in bulk drug and dosage forms

Chromatographic methods: A reversed phase high-performance liquid chromatographic method with detection at 220 nm was developed for tazobactam in tazocin injectable powder using an internal standard acetaminophen. A hypersil BDS RP-C_{18} column (250 mm × 4.6 mm), 5 μm particle size, was used with a mobile phase composed of aqueous solution of sodium dihydrogen phosphate dihydrate,acetonitrile and methanol in the ratio of 70:15:15 v/v/v[41]. An high performance liquid chromatography method for simultaneous estimation of piperacillin and tazobactam in injection formulations was developed using wakosil II, C_{18}, 250 mm × 4.6 mm, 5 μm column, with mobile phase composition of methanol, phosphate buffer and acetonitrile in the ratio of 1:2:1 v/v/v with the flow rate of 1 mL/min and UV detection at 220 nm[42].

Analysis in biological fluids: Serum and cerebrospinal fluid samples containing tazobactam was separated on a dual-column HPLC system (precolumn, RP 2, 10 μm; analytical column, spherisorb ODS II, 5 μm) using the mobile phase of sodium dihydrogen phosphate, tetrabutylammonium hydrogen sulfate and acetonitrile. The eluent from the column was detected at 210 nm[43]. Rapid determination of sulbactam and tazobactam in human serum by HPLC was reported using a Nova Pak column (150 × 4.3 mm, 5 μm) and the mobile phase of phosphate buffer and methanol in the ratio of 95:5. The detection wavelength used was 224.9 nm. It was also found that lower pH values of mobile phase excessively increased the analysis time[44]. Gradient elution high-performance liquid chromatographic method for the analysis of tazobactam simultaneously with piperacillin in human plasma, serum, bile and urine was reported. Separation and quantitation were achieved using a mobile phase based on ion-suppression chromatography on a C_{18} reversed-phase column with ultraviolet detection at 220 nm[45]. Pharmacokinetics studies were performed on Chinese volunteers for tazobactam using an improved HPLC method. The method uses a C_{18} column for separation with the mobile phase containing sodium dihydrogen phosphate and methanol in the ratio of 90:10 v/v. The detection wavelength used was 220 nm[46]. HPLC determination of tazobactam and piperacillin in human plasma and urine was carried out with UV detection at 218 nm. An internal standard ceftazidime was used for the analysis of plasma samples. A LiChrosorb RP-select B column with acetonitrile and ammonium acetate mobile phase was used[47]. Precolumn derivatization using 1,2,4-triazole was done to analyze tazobactam in serum and haemofiltration fluid by HPLC. After derivatization the absorbance maximum of the drug was found to be 325 nm. A Merck Lichrospher RP 18 column (125 × 4.6mm, 5 μm particle size) with diammonium hydrogen phosphate buffer and methanol in the ratio of 99.5:0.5 v/v was used as the mobile phase[6].

Sulbactam: Chemically it is (2S, 5R)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide[9,10]. It is a semi synthetic β lactamase inhibitor, derivative of the penicillin nucleus, is a penicillanic acid sulfone.

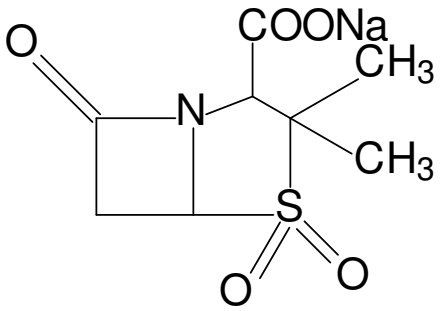

Fig. 4. Chemical structure of sulbactam sodium

It is a white crystalline solid with melting point 148-151 ºC[10]. It broadens the spectrum of penicicillins and cephalosporins by making it effective against organisms that express β-lactamase and would normally degrade penicicillins and cephalosporins.

Analysis in bulk drug and dosage forms

Spectroscopic methods: Three spectrophotometric methods have been reported for the simultaneous estimation of cefotaxime sodium and sulbactam sodium in pharmaceutical dosage forms. The first method involves determination using the simultaneous equation method, the sampling wavelengths selected are 233.5 and 264 nm and the second method is the area under curve method, the sampling wavelength ranges selected are 238.5-228.5 and 269-259 nm with linearity in the concentration ranges of 5-35 and 2.5-17.5 mcg mL^{-1} for

cefotaxime sodium and sulbactam sodium. The third method involves determination using the multicomponent mode method, the sampling wavelengths selected are 233.5 and 264 nm over the concentration ranges of 5-35 and 2.5-17.5 mcg mL^{-1} for cefotaxime sodium and sulbactam sodium respectively[48]. Fractional wavelet transform (FWT) was used for the simultaneous determination of ampicillin and sulbactam in a pharmaceutical combination for injection. This approach is a new powerful tool for removing noise and irrelevant information from the absorption spectra. Fractional wavelet transform approach was subjected to the data vector of the UV-signals obtained from ampicillin and sulbactam in the wavelength range of 211.5-313.8 nm. Derivative transform was applied to the original absorption signal together with its FWT generalization. Fractional wavelet transform approach was compared with the usual derivative spectrophotometry and chemometric methods and a good agreement was reported[49]. First and second derivative spectrophotometric assay is described to measure the concentrations of cefoperazone and sulbactam in injectable formulations. Since zero-order spectra are subject to interference, derivative spectrophotometry was used to enhance the spectral details in this method. Beer's law is obeyed up to 75 μg mL^{-1} of sulbactam in the second derivative mode[50]. A simple spectrophotometric method was used for the resolution of the binary mixtures of ampicillin sodium and sulbactam sodium. In aqueous solution, zero-order spectra are subjected to interference, so first-derivative spectrophotometry was used to enhance the spectral details allowing the determination of ampicillin sodium from the signal at the zero-crossing point for sulbactam sodium at 268 nm. In 0.1 N sodium hydroxide, sulbactam sodium was determined from the absorbance at 260 nm with negligible contribution from ampicillin sodium. Sulbactam sodium was also determined without interference using first- and second-derivative spectra in 0.1 N sodium hydroxide at 276 nm (peak-height) and 262-284 nm (peak-to-peak), respectively[51]. Two signal processing approaches, ratio spectra- first and ratio spectra-second derivative, were used for quantitative analysis of a mixture containing ampicillin sodium and sulbactam sodium in two different commercial pharmaceutical preparations for injection. The UV-absorption spectra of the analyzed drugs and their samples are recorded in the spectral range of 210-340 nm. These methods have the advantage that no prior separation step is required[52].

Chromatographic methods: Sulbactam was analyzed by HPLC along with ceftazidime in combined dosage form and in spiked plasma. Chromatographic separation of two drugs was achieved on a hypersil ODS C_{18} (250 × 4.6 mm, 5 μ) column using a mobile phase consisting of a binary mixture of acetonitrile and tetrabutyl ammonium hydroxide in ratio of 25:75 with the detector wavelength set at 230 nm[53]. Simultaneous determination of amoxicillin and sulbactam pivoxil in a combination formulation was carried out by liquid chromatography using hypersil C_{18} column (250 × 4.6 mm, 5 μm) and a mobile phase consisting of a mixture of methanol, acetonitrile and water in the ratio of 60:1:39. The mobile phase was delivered at a flow rate of 1.0 mL/min and detection was made at 220 nm[54]. A mobile phase of methanol and 0.01 M tetrabutylammonium hydroxide in the ratio of 50:50 v/v was used for the analysis of sulbactam sodium simultaneously with piperacillin sodium in sterile powder for injection. A Diamonsil C_{18} column (150 × 4.6mm, 5 μm) was used and the absorption was measured at 220 nm. Salicylic acid was used as internal standard in this method[55]. An isocratic liquid chromatographic method with UV detection at 230 nm is described for simultaneous determination of ceftriaxone sodium and sulbactam sodium in cetriax-s 1.5 gm injection. Chromatographic separations of two drugs was achieved on a hypersil ODS C_{18} column (250 mm × 4.6 mm, *i.d.*, 5 μm,) using a mobile phase consisting of potassium dihydrogen orthophosphate and acetonitrile in the ratio of 90:10 v/v[56]. Sulbactam was analyzed along with tazobctam and clavulanic acid in human sera using β-cyclodextrin column by high performance liquid chromatography. The mobile phase consisted of methanol and tetraethylammonium acetate in the ratio of 35:65 v/v and delivered at a flow rate of 1.0 mL/min[57]. An isocratic liquid chromatographic method with UV detection at 210 nm is described for simultaneous determination of amoxicillin sodium and sulbactam sodium in a new combination formulation using a hypersil C_{18} column. The commonly used ion-pairing reagent was not used in this method, instead a simple mobile phase consisting of a binary mixture of methanol and sodium acetate in the ratio of 5:95 v/v was used[58]. Simultaneous determination of ampicillin sulbactam and cefoperazone in pharmaceutical formulations by using HPLC with β-cyclodextrin stationary phase was developed with the help of tetraethylammonium acetate buffer and methanol in the ratio of 65:35 v/v as a mobile phase. The detection wavelength was selected at 280 nm[59]. Reversed-phase ion pair high performance liquid chromatography was employed for determining the content of mezlocillin sodium and sulbactam sodium in a formulation meant for injection. A bondapak C_{18} column (250 mm × 4.6 mm, 10 μm) and a mobile phase consisting of tetrabutylammonium hydroxide and acetonitrile in the ratio of 68:32 v/v with UV detection at 230 nm was used[60]. An HPLC method for determination of related substances in sulbactam sodium was developed using a C_{18} column and tetrabutylammonium hydroxide and acetonitrile in the ratio of 75:25 v/v as the mobile phase. The flow rate of the mobile phase was maintained at 1 mL/min and the detection wavelength was set at 230 nm. The method was successfully used for quality control of sulbactam sodium[61].

Analysis in biological fluids: Analysis of sulbactam was performed in plasma using imidazole as a derivatizing agent to increase the sensitivity of the method and metronidazole as a internal standard to increase the accuracy of the method. TSK gel super ODS (50 mm × 4.6 mm i.d., 2 μm) column with methanol and phosphate buffer with tetrabutylammonium hydroxide solution was used as a mobile phase. The column eluent was detected at 313 nm[62]. A gradient liquid chromatographic method with UV detection at 230 nm is described for simultaneous determination of rifampicin and sulbactam in mouse plasma. Chromatographic separation was achieved on a RP-18 (125 mm × 4 mm, 5 μm) column with potassium dihydrogen phosphate solution and acetonitrile at a flow-rate of 1.0 mL/min as a mobile phase[63]. Using borate buffer and methanol in a gradient run within 11 min simultaneous determination of imipenem and sulbactam in mouse plasma was completed. In this method detection wavelength is set at 230 nm for sulbactam when analyzed with a C_{18} column[64].

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