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Review article

Review on determination of cefepime in biological fluids by different analytical methods

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Abstract

Cefepime is one of the important fourth generation cephalosporins used in the management of infections because of its potent and broad-spectrum antimicrobial activity. There are many methods for its analysis also alone or in combination with other drugs in serum, cerebrospinal fluid and urine. Some of those methods are Spectrophotometric, HPLC alone or with MS, HPLC with UV, Electrophoresis and Micellar Electrokinetic Chromatography with UV. This article reviews the different analytical methods of cefepime in the biological fluids.

Key words: Cefepime, Chromatography, Spectrophotometry, Electrophoresis, Serum, Biological fluids.

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1. Introduction

Cephalosporins with antimicrobial, pharmacological and toxicological effects have been developed. One of them, cefepime, is a parenteral cephalosporin with low affinity and good stability for extended spectrum β-lactamase. The determination of this drug in therapeutic monitoring is of particular interest because of its activity. It represents an improvement in the β-lactam group antibiotics and is considered the therapy of choice for a variety of infections that develop in intensive care units [1]. This paper is reviewed the analytical methods for determination of cefepime in biological fluids from year 2000 to 2015.

The chemical name of cefepime is 7-[α-(2-aminothiazol-4-yl)-α-(z)methoxyimino acetamido]-3-(1-methylpyrrolidino)-methyl-3-cephem-4 carboxylate, it is characterized by the presence of a positively charged quaternerized N-methyl-pyrrolidine substitution at the 3 position of the cephem moiety, making cefepime a zwitter ion as shown in Figure 1. It is official in The United States Pharmacopeia and the British Pharmacopeia. It is used clinically for the treatment of lower respiratory tract, intra-abdominal, urinary tract, skin and soft tissue infections and also used for prophylaxis in biliary tract and prostate

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surgery. Cefepime/TAZ is one of the dosage form combination already licensed and used in Indian hospitals [2, 3].

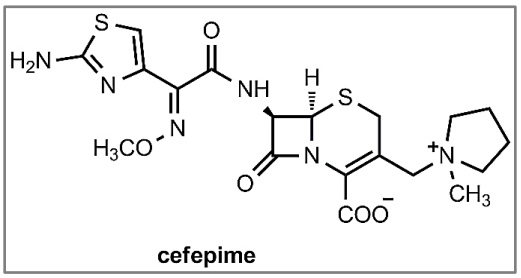

Figure 1: Structure of cefepime

2. Methods of analysis:

Spectroscopic methods:

Abdel-Aziz O et al. [4] have been developed a method for simultaneous determination of cefepime and levofloxacin (co-administered dosage form) in human plasma by applying, two techniques, one of them is Savitzky–Golay differentiation filters which is based on derivatization of the absorbance spectra of both drugs and then obtaining the first derivative by calculating the different coefficients of each drug using the least-squares polynomial equation. The ratio spectrum is obtained by plotting the mean of the wavelengths set and so the peak amplitude of the obtained spectrum is directly proportional to the concentration of single analyte in the mixture. The second one is a combined trigonometric Fourier functions to their ratio spectra that is based on the determination of single compound by calculating Fourier function ratio spectrum coefficients from a set of absorbance ratios. The drug's stock solutions are prepared in water and the plasma samples are extracted with methanol. The amplitudes were measured at (272, 336nm), (266, 322nm) for cefepime and levofloxacin in the first and the second methods, respectively.

Chromatographic methods

Different chromatographic techniques were developed alone or with other techniques for analysis of cefepime.

Ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS):

Cazorla RR et al. [5] used ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS) for the simultaneous analysis of 21 antibiotics in urine, serum, cerebrospinal fluid and bronchial aspirations. The method based on the centrifugation of samples from human biological fluids at 4500 rpm for 10 min and then stored. The chromatographic conditions were optimized to obtain good separation and the chromatographic analysis was performed using an Acquity UPLC system. Mass spectrometry analysis was carried out using a mass spectrometer (Waters Acquity TQD tandem quadrupole). The separation in HPLC was done using an Acquity UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 m particle size). The samples were separated by gradient elution using mobile phase consisting of methanol (eluent A) and an aqueous solution of formic acid, 0.01%, v/v (eluent B), at a flow rate of 0.3 mL/min with running time < 6 minutes. Another method with the same technique was developed and qualified for the simultaneous determination of penicillins and cephalosporins in human plasma. Plasma samples were prepared for UPLC–MS/MS analysis by mixed-mode solid phase extraction. Chromatographic separation was achieved on an Acquity UPLC system equipped with an Acquity HSS T3 column (50mm, 2.1 mm, 1.7 mm particle size) and an Acquity BEH C18 guard-column (5mm, 2.1 mm, 1.7 mm particle size) using mobile phases consisted of a 1mM

CH_3COOH/CH_3COONH_4-buffer with 5% acetonitrile (MPA) and acetonitrile (MPB). Components were eluted using gradient-elution at a flow rate of 0.600 ml/min using five stable isotopes as internal standards. The MS/MS instrument was operated with a capillary voltage of 3.5 kV, source block temperature of 150 1C and cone voltage of 10 V. Nitrogen was used as desolvation gas [6].

Solid Phase Extraction HPLC:

Cefepime in serum was determined by solid phase extraction HPLC by Chen Y et al. [7]. The method based on the extraction of serum by a solid phase extraction cartridge using Waters Symmetry shield RP18 (5 μm, 250 mm× 4.6 mm) column. The mobile phase was eluted and detected at 254 nm with flow rate 1.0 ml/min using methanol-20 mmol/L ammonium acetate solution (13[DK]:87). The retention time of cefepime was 6.82 min and the linearity was obtained in the range of 0.5- 100.0 mg/L. The quantitation (LOQ) and RSD for within and between day were 4.29%, 9.83% and 5.21%, 10.57%, respectively.

High-Performance liquid chromatography (HPLC):

Four HPLC methods for analysis of cefepime were developed. The first method was based on determination of cefepime and ceftazidime in plasma and dialysate-ultrafiltrate samples obtained from intensive care unit (ICU) patients. The two drugs were prepared in water for plasma calibration but in saline for dialysate-ultrafiltrate, the plasma samples centrifuged with acetonitrile for 5 minutes and then with dichloromethane before injection. Separation was performed on a Bondapak C18 (30cm×3.9mm×10m) with UV detection. The mobile phase consists of acetate buffer: ACN and was delivered at 2 ml/min at wavelength 257 and 280 nm for ceftazidime and cefepime, respectively [8]. The second method was applied by Nemutlu E et al. [9] for the separation of seven cephalosporins in human plasma. All cephalosporins including cefepime were prepared in water except cefixime was prepared in methanol, the plasma and amniotic fluid were obtained and prepared as mentioned previous in the first method. The chromatographic conditions were optimized and the results were obtained using XTerra C18 (250mm ×4.6mm, 5m i.d.) column, (40mM phosphate buffer, pH 3.2, 18% MeOH) was used as a mobile phase with flow rate 0.85ml/min and 32◦C column temperature. Gradient elution with MeOH was applied. Cefixime and ceftizaxime were monitored at 285nm while cefoperazone and cefepime at 260nm.

The third method was developed by Palacios FJ et al., [10] involved the detection of cefepime at 256 nm in biological matrices. Serum samples were deproteinized with acetonitrile and extracted once with dichloromethane except urine and cerebrospinal fluid samples need microfiltration. The type of HPLC equipment is (Merck–Hitachi–Lachrom, Spain) which comprised of an L-7100 pump, a LiChrospher 100 RP-18 column (250 mm 4 mm, 5 lm particles), and a L-7455 diode-array detector. The injector was a Rheodyne (model 7725i) manual injection valve. A good separation was obtained using pH 7 phosphate buffer 10 mM–methanol, (75:25) as a mobile phase. Flow-rates were 1ml/min for detection of cefepime in serum and urine and 0.5ml/min for its detection in cerebrospinal fluid. UV detection was performed at 256 nm while the fourth method was RP-HPLC-UV with solid phase extraction which applied by Iqbal MS et al. [11] for the determination of cefepime in total nutrient admixtures.

High-Performance liquid chromatography and Spectro-photometry (HPLC with UV):

López KV et al. [12] have been proposed a liquid chromatographic method with UV-detection for simultaneous determination of three cephalosporins (cefepime, vancomycin and imipenem) in plasma. The samples protinized then they were injected. The chromatograph and peaks were recorded by SulpelcosilTM LC-18 column (25 cm×4.6 mm×5µm) and using Cefuroxime as internal standard. The mobile phase consisting of freshly prepared 0.075 Macetate buffer: acetonitrile (92:8v/v), pH 5.0 at flow rate (0.8ml/min). Optimization of factors affecting the separation was achieved with detection at wavelength 230 nm and the plasma samples were prepared by centrifugation at 6000 rpm at 4◦C for 40 min while Verdier MC et al. [13] applied the same previous method for the determination of 12 beta-lactam antibiotics (cloxacillin, imipenem, meropenem, oxacillin, penicillin G, piperacillin, and ticarcillin amoxicillin, cefepime, cefotaxime, ceftazidime, ceftriaxone) in human plasma samples simultaneously. The samples also were protinized as the first method but the separation was performed using an Atlantis T3 analytical column (150 by 4.6 mm, 5µm; Waters), coupled with an Atlantis T3 guard column. The chromatogram was obtained using linear gradient of acetonitrile and a pH 2 phosphoric acid solution with a flow rate of 2ml/min. The running time was 22 min. The mentioned drugs were prepared in water while the plasma sample were centrifuged for 10 min at 3,000 rpm and 4°C wavelength detection was set either at 210, 230, or 298 nm according to the compound.

Liquid chromatography with Mass spectrometry (LC-MS):

Ohmori T et al. [14] proposed a liquid chromatography technique with mass spectrometry for the determination of 8 antibiotics in human serum in patients admitted to the intensive care unit (ICU) simultaneously. The antibiotics were prepared in water while IS was prepared in methanol and the serum samples were extracted with 10mM ammonium formate and then loaded to Oasis® HLB cartridges. Chromatographic separation was achieved with UK-C18 (3µm reversed-phase porous ODS, 50 mm×2 mm I.D) and under gradient elution using a mobile phase consisting of 10m Maqueous ammonium formate containing 0.1% formic acid (eluent A) and methanol containing 0.1% formic acid (eluent B) in the ratio (95:5) at a time from 0 to 0.5 minutes. The flow rate of running was set at 0.3ml/min. The conditions for MS technique were that 3.5kV voltage using Nitrogen as desolvation gas.

Micellar electrokinetic chromatography with UV:

Four methods were described for analysis of cefepime in biological fluids by micellar electrokinetic chromatography with UV, in the first method cefepime and vancomycin are separated using BGE consisting of buffer with SDS and methanol as electrolyte solution [15]. The second method is described for analysis of cefepime in plasma and cerebrospinal fluid by direct injection without any sample pretreatment. The separation was performed using electrolyte consisting of tris(hydroxymethyl)aminomethane buffer with sodium dodecyl sulfate (SDS) as the electrolyte solution using cefazolin as an internal standard [16]. Several parameters affecting the separation of the drug in each method were studied, including the pH and concentrations of the

buffer and SDS also cefepime was determined in human serum [17] directly by micellar electrokinetic capillary chromatography.

Capillary zone electrophoresis (CZE):

The capillary zone electrophoresis was applied by Andrási M et al. [18] for the analysis of cephalosporins in bronchial serum and sputum. The instrument used is HP 3DCE model and the buffer electrolyte of pH 9.1 included 25 mM borate and 50 mM SDS. The serum and sputum samples were collected from the patients and injected directly to the capillary in presence of serum but pretreatment with water or methanol is required with sputum. The obtained good recovery data prove that the lyophilization/dissolution of bronchial secretion samples can be reproducibly performed. The samples were recorded at wavelength 270 nm.

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