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Analytical method development and validation of ceftazidime and avibactam by UV visible spectroscopy and RP- HPLC

Monika R Dumbre, Aishwarya P Walke, Shreyash P Vyavahare, Pranav S Pawar, Shubham U Gholap
Students, Samarth College of Pharmacy, Belhe, Pune, Maharashtra, India

Abstract
Ceftazidime-avibactam is an intravenously administered combination of the third-generation cephalosporin ceftazidime and the novel, non-β-lactam β-lactamase inhibitor avibactam. ceftazidime-avibactam is approved for the treatment of adults with complicated urinary tract infections, complicated intra-abdominal infections, hospital-acquired pneumonia (HAP), and other infections caused by aerobic. This article discusses the *in vitro* activity and pharmacological properties of ceftazidime-avibactam, and reviews data on the agent's clinical efficacy and tolerability relating to use in these indications. Ceftazidime-avibactam has excellent *in vitro* activity against many important Gram-negative pathogens, including many extended-spectrum. It is not active against metallo-β-lactamase-producing strains. Ceftazidime-avibactam treatment was associated with high response rates at the test-of-cure visit in patients with infections caused by ceftazidime-susceptible and - nonsusceptible Gram-negative pathogens. Ceftazidime-avibactam was generally well tolerated, with a safety and tolerability profile consistent with that of ceftazidime alone and that was generally typical of the injectable cephalosporins. Thus, ceftazidime-avibactam represents a valuable new treatment option for these serious and difficult-to-treat infections.
Objective: Ceftazidime/avibactam resistance due to mutation in the omega loop of KPC-2 has been documented *in vitro* and *in vivo.* This study evaluated the mechanism of ceftazidime/avibactam resistance in a KPC-2-expressing Klebsiella pneumoniae isolated from a patient following ceftazidime/avibactam combination therapy with gentamicin for the treatment of ventilator-associated pneumonia.

Keywords: ceftazidime, avibactam

Introduction
The increasing prevalence of multidrug resistant (MDR) Gram-negative bacterial pathogens worldwide is a significant global public health concern. Antimicrobial resistance among Gram-negative pathogens (in particular, resistance to β-lactam antimicrobials) is commonly driven by the production of β-lactamases, which can greatly limit treatment options for serious bacterial infections. The increasing prevalence of extended-spectrum β-lactamase (ESBL)-producing pathogens has driven increased use of and reliance on carbapenems. The emergence and spread of carbapenemase producing pathogens (including arbapenem-resistant Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacteriaceae) is thus of particular concern and has highlighted the urgent need for new antimicrobial agents.

Table1: PICOS method for selecting clinical studies in the systematic reviews.

Participans	Intervention	Comparison	Outcomes	Study Design
Adult patients in any setting with confirmed bacterial infection	Ceftazidime avibactam in association with another antibiotic	Ceftazidime avibactam alone	Primary Outcomes: all cause mortility Secondary Outcomes: a) not clinical improvement, b) not microbiological improvement	Randomized controlled trials and observational studies

Effectiveness of ceftazidime/avibactam
1. Methods
We designed an observational study of a prospectively collected cohort of adult patients receiving ceftazidime/avibactam in our centre. Only the first treatment course of each patient was analysed. Efficacy and safety were evaluated as 14 and 30 day mortality, recurrence rate at 90 days, resistance development and occurrence of adverse effects.

2. Materials and methods
2.1 Materials
Ceftazidime, Avibactum, Avycaz (2000mg Ceftazidime + 500mg of Avibactum) Intravenous Infusion, distilled water, glacial acitic acid, methanol, potassium dihydrogen acetonitrile, phosphate buffer, ammonium acetate buffer, phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid all are from Rankem chemicals.

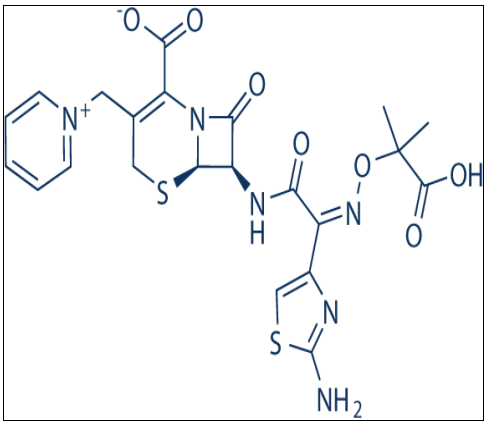
2.2 Instrument
HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Ceftazidime, Avibactum solutions.

2.3 Standard preparation
(400µg/ml Ceftazidime & 100µg/ml Avibactum) Accurately Weighed and transferred 40mg&10mg of Ceftazidime and Avibactum working Standards into a 10ml and 10ml clean dry volumetric flask respectively, add 7ml and 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1ml

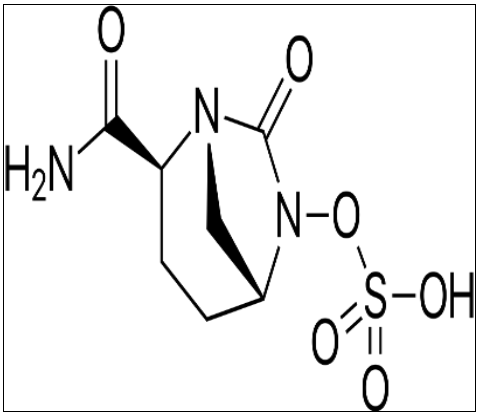
was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluent.

2.4 Sample preparation

5 bottles were weighed and calculate the average weight of each bottle then the weight equivalent to 1 bottle was transferred into a 500 ml volumetric flask, 300ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.



Ceftazidime



avibactam

Limit of detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

- Based on Visual Evaluation.
- Based on Signal-to-Noise.
- Based on Standard Deviation of the Response and the Slope:
  LOD can be expressed as: LOD = 3.3σ/S

Where

σ = Standard deviation of intercepts of calibration curves,

S= Mean of slopes of the calibration curves

The slope S may be calculated from the calibration curve of the analyte.

Limit of quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Several approaches for determining the Quantitation limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

- Based on Visual Evaluation
- Based on Signal-to-Noise
- Based on Standard Deviation of the Response and Slope
  LOQ can be expressed as**:** LOQ = 10σ/S

Where

σ= Standard deviation of intercepts of calibration curves.

S= Mean of slopes of the calibration curves.

The slope S may be calculated from the calibration curve of the analytical range. The range of an analytical procedure is the interval between the upper and lower concentration for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The range of the analytical procedure is validated by verifying that the analytical procedure provides acceptable precision, accuracy and linearity when applied to the samples containing analytes at the extremes of the range as well as within the range.

Storage conditions

a. Prior to reconstitution: Protect from light. Store at 15-300° C
b. After reconstitution: Store in a refrigerator and use within 7 days. If kept at room temperature, use within 24 hours.
c. Once reconstituted, light protection is not needed.

Chemicals

Acetonitrile HPLC grade, distilled water and anhydrous sodium acetate analytical grade are procured from scharlau, Spain.

Mobile phase preparation

Acetate Buffer pH (7.0): Acetonitrile (60:40) Sodium acetate buffer is prepared by dissolving 1.64gm of anhydrous sodium acetate in 700ml distilled water, sonicate to dissolve and adjust pH to 7.0 by ortho phosphoric acid solution. Make up to 1000 mL with distilled water, filter and degass mixtures of buffer and acetonitrile through 0.45µ membrane filter under vacuum pump. Diluent Sodium acetate buffer pH (7.0)

Construction of calibration curves

Different concentrations of CFZ and PY equivalent to (100–400) µg /mL and (5-50) µg /mL for CFZ and PY, respectively, are separately withdrawn from their respective stock standards into separate series of 100 mL volumetric flasks, and the volumes are made up to volume with the diluent. Duplicate 20 µL injections are made for each concentration maintaining the flow rate at 1.5mL/min and the effluent is UV-scanned at 254 nm. The chromatographic separation is performed following the procedure under chromatographic conditions. The chromatograms are recorded, peak areas of CFZ and PY are determined and the

calibration curves relating the obtained integrated peak areas to the corresponding concentrations are constructed and the regression equations are performed.

Results and discussion

The aim of this work is to introduced simple, sensitive, accurate, precise and smart RP-HPLC method for simultaneous estimation of clofazimine (CFZ) and its impurity product in powder used for making solution in vial for IM & IV injections. Also, to determine the concentration and recovery for laboratory prepared mixtures and subjecting them to the standard addition technique. Chromatograms are obtained with significantly different RF values of 1.456 and 2.970 min for CFZ and Pyridine (PY) with correlation coefficient (r) >0.9999, limits of detection 3.40 and 0.16 µg mL-1 and limits of quantization of 10.33 and 0.49 µg mL-1 for CFZ PY respectively. No occurrence of interfering peaks has been detected.

Conclusion

The proposed RP-HPLC method for simultaneous estimation of Ceftazidime pentahydrate and its impurity product Pyridine in powder used for making solution in vial for IM & IV injections is novel, precise, specific, accurate, less time consuming, low cost and rapid. Based on the results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines, stability of tested drugs is evaluated. The method can be used for regular routine analysis and stability study. However, further studies are needed in order to determine the sodium carbonate content in combination with both drugs in powder used for making solution in vial for IM & IV injections.

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