INTRODUCTION

Ceftazidime chemically is 1-{[(6R,7R)-7-[(2Z)-2-(2-amino-1, 3-thiazol-4-yl) imino] acetamido] -2- [1-carboxy-1-methylethoxy) pyridin-1-ium} the bactericidal activity of ceftazidime results from the inhibition of cell wall synthesis via affinity for penicillin-binding proteins (PBPs) [1-2]. Avibactum chemically is sodium (2S, 5R)-2-carbamoyl-7-oxo-1, 6-diazabicyclo [3.2.1] octan-6-yl sulfate. Avibactam is a non-β lactam β-lactamase inhibitor that inactivates some β-lactamases (Ambler class A β-lactamases, including Klebsiella pneumoniae carbapenemases).

Ambler class C and some Ambler class D β-lactamases) by a unique covalent and reversible mechanism, and protects ceftazidime from degradation by certain β-lactamases [3-4].

Literature survey reveals there are analytical methods developed for simultaneous estimation of caftazimide either individually [5-6] or in combination with other drugs in tablet dosage form [7-9] or in plasma [10] but not in combination with Avibactum. Hence an attempt is made in order to develop a new method for simultaneous estimation of Ceftazidime and Avibactum in tablet dosage form. The proposed method was validated according to ICH guidelines [11-12].

SIMULTANEOUS ESTIMATION OF CEFTAZIDINE AND AVIBACTUM IN TABLET DOSAGE FORM BY RP-HPLC

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Abstract

A simple and selective LC method is described for the determination of Ceftazidime and avibactum in tablet dosage forms. Chromatographic separation was achieved on a c18 column Inertsil ODS, (250×4.6× 5µ) using mobile phase consisting of a mixture of Phosphate buffer and Acetonitrile(60:40), P4-H4 ,with detection of 231nm. The retention times were 2.523mins and 4.410mins for Ceftazidine and Avibactum respectively. Linearity was observed in the range 6-14 µg/ml for Ceftazidine (r²=0.995) and 6-14 µg /ml for Avibactum (r²=0.999).

The proposed method was validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: RPHPLC, CEFTAZIDINE, AVIBACTUM.
EXPERIMENTAL SECTION: The instrument employed for present study is as follows

| Table No 1: INSTRUMENT EMPLOYED |
|--------------------------------|
| UV-Visible Spectrophotometer   | Nicolet evolution 100 |
| UV-Visible Spectrophotometer software | Vision Pro |
| HPLC software                  | Spin chrome (LC SOLUTIONS) |
| HPLC                           | Shimadzu(LC 20 AT VP) |
| Ultra sonicator                | Citizen, Digital Ultrasonic Cleaner |
| pH meter                       | Global digital |
| Electronic balance             | Shimadzu |
| Syringe                        | Hamilton |
| HPLC Column                    | Inertsil ODS 3V(250x4.6mm) 5μm |

The reagents used in the present study are listed in table 2.

| Table No 2: REAGENTS USED |
|----------------------------|
| Water                      | HPLC Grade |
| Potassium Phosphate        | AR Grade   |
| Acetonitrile               | HPLC Grade |
| Ammonium acetate           | AR Grade   |
| Disodium hydrogen phosphate | AR Grade  |

Drugs used in the present study are listed in table 3

| Table No 3: Drugs used in the present study |
|--------------------------------------------|
| Ceftazidime and Avibactum standards        | Gift Samples obtained from Chandra labs, Hyd. |
| Ceftazidime(2gm) & Avibactum (0.5gm) (label claims). | Obtained from local pharmacy |

CHROMATOGRAPHIC CONDITIONS:

Mobile Phase:
The mobile phase used was a mixture of Phosphate buffer and acetonitrile pH-4.0 in the ratio of 60:40 v/v; it was filtered before use through a 0.45 μm membrane filter and degassed for 30 min. The elution was carried out isocratically at the flow rate of 1.0 ml/min. Detection was carried out at 231 nm at ambient temperature.

Preparation of buffer:
28.8 gm of potassium di hydrogen phosphate (KH₂PO₄) was weighed and dissolved in 100ml of water and volume was made up to 1000ml with water. Adjust the pH to 6.8 using ortho phosphoric acid. The buffer was filtered through 0.45μ filters to remove all fine particles and gases.

Preparation of standard stock solution of CEFTAZIDIME
10 mg of CEFTAZIDIME was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 μg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of AVIBACTAM
10 mg of AVIBACTAM was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 μg /ml of solution by diluting 1ml to 10ml with methanol.

ISOBESTIC POINT OF CEFTAZIDIME AND AVIBATAM:
The wavelength of maximum absorption (λ_max) of the drug, 10 μg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The isobestic point was found to be 231 nm for the combination.

OPTIMISATION OF CHROMATOGRAPHIC CONDITIONS:

Preparation of mixed standard solution
Weigh accurately 2.0 gms of CEFTAZIDIME and 0.5gms of AVIBACTAM in 100 ml of volumetric flask and dissolve in 100ml of mobile phase and make up the volume with mobile phase. From above stock solution 25000 μg/ml of CEFTAZIDIME and AVIBACTAM is prepared by diluting 5 ml to 50ml with mobile phase.
Figure No 1: Chromatogram of AVIBACTAM and CEFTAZIDIME

TABLE No 4: ASSAY RESULTS

|                  | CEFTAZIDIME | AVIBACTAM |
|------------------|-------------|-----------|
|                  | Standard Area | Sample Area | Standard Area | Sample Area |
| Injection-1      | 2334.362     | 2344.463   | 207.967       | 212.684     |
| Injection-2      | 2323.199     | 2351.614   | 199.698       | 209.655     |
| Injection-3      | 2337.863     | 2337.863   | 207.039       | 207.039     |
| Injection-4      | 2331.502     | 2334.732   | 207.039       | 207.039     |
| Injection-5      | 2328.483     | 2341.801   | 207.039       | 207.039     |
| Average Area     | 2336.588     | 2342.095   | 205.1066      | 209.91      |

|                  | Standard deviation | % RSD | Assay(%)purity |  |
|------------------|--------------------|-------|----------------|---------|
|                  | 6.489006           | 0.276506 | 100.23         | 102.21  |

The amount of CEFTAZIDIME and AVIBACTAM present in the taken dosage form was found to be 100.23 % and 102.21% respectively.
### Table No 5: Results for system suitability of CEFTAZIDINE

| Injection | Retention time (min) | Peak area       | Theoretical plates (TP) | Tailing factor (TF) |
|-----------|----------------------|-----------------|-------------------------|---------------------|
| 1         | 2.523                | 2334.362        | 3304                    | 1.308               |
| 2         | 2.523                | 2323.199        | 3304                    | 1.308               |
| 3         | 2.520                | 2337.863        | 3295                    | 1.400               |
| 4         | 2.523                | 2331.502        | 3304                    | 1.308               |
| 5         | 2.520                | 2328.583        | 3295                    | 1.400               |
| **Mean**  | 2.5218               | 2331.102        | -                       | -                   |
| **SD**    | 0.001643             | 5.596907        | -                       | -                   |
| **% RSD** | 0.065028             | 0.239617        | -                       | -                   |

### Table No 6: Results for system suitability of AVIBACTUM

| Injection | Retention time (min) | Peak area       | Theoretical plates | Tailing factor |
|-----------|----------------------|-----------------|--------------------|----------------|
| 1         | 4.417                | 207.967         | 3304               | 1.308          |
| 2         | 4.417                | 199.698         | 7105               | 1.161          |
| 3         | 4.403                | 207.039         | 7460               | 1.156          |
| 4         | 4.417                | 207.632         | 7505               | 1.156          |
| 5         | 4.403                | 198.197         | 7406               | 1.194          |
| **Mean**  | 4.4114               | 204.1066        | -                  | -              |
| **SD**    | 0.007668             | 4.751032        | -                  | -              |
| **% RSD** | 0.173477             | 2.323066        | -                  | -              |

**Figure No 2: Blank chromatogram for specificity by using mobile phase**
From the above stock solution 1000µg/ml of CEFTAZIDIME and AVIBACTUM is prepared by diluting 1ml to 10 ml with mobile phase (10µg/ml). This solution is used for recording chromatogram.

**ASSAY:**

**Preparation of mixed standard solution:** Weigh accurately 2.0 gm of CEFTAZIDINE and 0.5 gms of AVIBACTAM in 100 ml of volumetric flask and dissolve in 100ml of mobile phase and make up the volume with mobile phase. From above stock solution 25000 µg/ml of CEFTAZIDIME and AVIBACTAM is prepared by diluting 5ml to 50ml with mobile phase. The above stock solution 1000µg/ml of CEFTAZIDIME and AVIBACTUM is prepared by diluting 1ml to 10 ml with mobile phase (10µg/ml). This solution is used for recording chromatogram.

**Table No 7: Linearity Preparations**

| Preparations | Volume from standard stock transferred in ml | Volume made up in ml (with mobile phase) | Concentration of solution(µg /ml) |
|--------------|---------------------------------------------|------------------------------------------|---------------------------------|
| Preparation 1 | 0.6                                         | 10                                       | 60                             |
| Preparation 2 | 0.8                                         | 10                                       | 80                             |
| Preparation 3 | 1.0                                         | 10                                       | 100                            |
| Preparation 4 | 1.2                                         | 10                                       | 120                            |
| Preparation 5 | 1.4                                         | 10                                       | 140                            |

**Table No 8: linearity of CEFTAZIDINE**

| S.No. | Conc.(µg/ml) | Area       |
|-------|--------------|------------|
| 1     | 60           | 1344.606   |
| 2     | 80           | 1849.853   |
| 3     | 100          | 2338.421   |
| 4     | 120          | 2563.186   |
| 5     | 140          | 3106.591   |

**Table No 9: linearity of AVIBACTUM**

| S.No. | Conc.(µg/ml) | Area       |
|-------|--------------|------------|
| 1     | 60           | 108.783    |
| 2     | 80           | 159.306    |
| 3     | 100          | 204.849    |
| 4     | 120          | 222.682    |
| 5     | 140          | 243.873    |

**Accuracy:** Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%, 150%. Recovery results for AVIBACTUM

**Observation**

The percentage mean recovery of CEFTAZIDINE and AVIBACTUM is 100.10% and 99.75% respectively.
Table No 8: Recovery results for Avibactum

| Recovery level | Amount taken(mcg/ml) | Area | Average area | Amount recovered(mcg/ml) | %Recovery |
|----------------|----------------------|------|--------------|-------------------------|-----------|
| 50%            | 10                   | 236.388 | 237.6637     | 9.56                    | 92.65     |
|                | 10                   | 234.158 |              |                         |           |
|                | 10                   | 242.445 |              |                         |           |
| 100%           | 15                   | 224.953 | 254.704      | 15.03                   | 99.34     |
|                | 15                   | 269.243 |              |                         |           |
|                | 15                   | 269.916 |              |                         |           |
| 150%           | 20                   | 280.051 | 275.7967     | 21.07                   | 107.35    |
|                | 20                   | 280.286 |              |                         |           |
|                | 20                   | 267.053 |              |                         |           |

Figure No 3: Linearity graph of CEFTAZIDINE

Figure No 4: Linearity graph of Avibactum

\[y = 21.187x + 121.88\]
\[R^2 = 0.997\]

\[y = 1.5388x + 33.441\]
\[R^2 = 0.9995\]
Table No 9: Recovery results for CEFTAZIDINE

| Recovery level | Accuracy CEFTAZIDINE | Average % Recovery |
|----------------|----------------------|--------------------|
|                | Amount taken (mcg/ml) | Area               | Average area | Amount recovered | %Recovery |
| 50%            | 10                   | 2608.241           | 2607.639     | 10.8             | 111.3     |
|                | 10                   | 2609.160           |               |                  |           |
|                | 10                   | 2605.517           |               |                  |           |
| 100%           | 15                   | 2194.643           | 2244.257     | 13.25            | 95.85     |
|                | 15                   | 2111.816           |               |                  | 100.10    |
|                | 15                   | 2326.313           |               |                  |           |
| 150%           | 20                   | 3109.681           | 3109.702     | 22.5             | 93.16     |
|                | 20                   | 3112.744           |               |                  |           |
|                | 20                   | 3106.682           |               |                  |           |

Table No 10: Results for Method precision of CEFTAZIDINE and AVIBACTUM

| Parameter       | CEFTAZIDINE          | AVIBACTUM          |
|-----------------|----------------------|--------------------|
|                 | Retention time (min) | Tailing factor     | Retention time (min) | Tailing factor |
| Flow Rate       |                       |                    |                       |                |
| 0.8 ml/min      | 3.130                 | 1.258              | 5.443                 | 1.167          |
| 1.2 ml/min      | 2.090                 | 1.036              | 3.663                 | 0.943          |
| Wavelength      |                       |                    |                       |                |
| 229nm           | 2.513                 | 1.222              | 4.380                 | 1.088          |
| 233nm           | 2.517                 | 1.179              | 4.380                 | 1.125          |
| avg             | 2.513833             | 4.403333           | 198.551               |
| stdev           | 0.010926             | 0.009893           | 213.8968              |
| % RSD           | 0.433746             | 0.224216           | -                     |

Table No 11: Result of Robustness study

| Parameter       | CEFTAZIDINE | AVIBACTUM | |
|-----------------|-------------|-----------|
|                 | % Assay     | % Assay   | |
| Analyst 01      | 99.36       | Analyst 01| 96.28   |
| Analyst 02      | 99.30       | Analyst 02| 95.97   |

From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged.
Precision
Method precision
Prepared sample preparations of AVIBACTUM and CEFTAZIDINE as per test method and injected 6 times in the column.

Acceptance criteria
The % Relative standard deviation of Assay preparations of AVIBACTUM and CEFTAZIDINE should be not more than 2.0%.

Observation
Test results for AVIBACTUM and CEFTAZIDINE are showing that the %RSD of Assay results are within limits.

Robustness
Chromatographic conditions variation
To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Ruggedness
The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts Results for Ruggedness

CONCLUSION
An attempt is made to develop a simple, cost effective, robust, Accurate and Precise analytical method for simultaneous estimation of Ceftazidime and Avibactum in tablet dosage form. The method was accurate and precise as RSD obtained was less than 2%.The proposed method was estimated for its linearity and range and Regression coefficient was 0.999 for both the drugs. The method was validated for all validative parameters according to ICH guidelines including ruggedness and robustness and the results were satisfactory and within the limits. Hence the proposed method can be used for routine analysis of Ceftazidime and Avibactum in pharmaceutical preparations.

REFERENCES
1. Annonymos:http://www.drugbank.ca/drugs/DB00438/Ceftazidime. [Cited: March 2016]
2. Annonymos:https://en.m.wikipedia.org/wiki/ceftazidime. [Cited: March 2016]
3. Annonymos:www.drugbank.ca/drugs/DB09060/Avibactam. [Cited: March 2016]
4. Annonymos:https://en.m.wikipedia.org/wiki/avibactam. [Cited: March 2016]
5. Zajac M, Jelinska A, Sobczak A, Musial W. Stability of ceftazidime pentahydrate in medicinal preparations biotm and ceftium. Acta poloniae pharmaceutical and drug research. 2004;62(1):11-15.
6. Arcelloni C, Basile M, Vaiani R, Bonini P, Paroni R. Determination of Ceftazidime concentration in Mueller Hinton agar by high-performance liquid chromatography. J Chromatogr A. 1996;742(1-2):121-126
7. Amareswari S, Nandakishore A, Aasif M, Khan S. Stability indicating RP-HPLC method for the estimation of ceftazidime pentahydrate and tazobactam sodium in bulk and dosage forms. Ind J Res Pharm Biotech. 2013;1(4):543-548.
8. Nanda R, Shelke A. Development and validation of RP_HPLC method for the simultaneous estimation of ceftazidime sodium and tazibactam sodium in marketed formulation. Int J Pharm Res. 2013;5(3):983-999.
9. Reddy J, Ganapaty S. A validated stability indicating RP-HPLC method for simultaneous determination of tobramycin and ceftazidime in pharmaceutical formulation. Int J Pharm. 2015;5(3):976-984.
10. Siddiqui MR, Tariq A, Chaudhary K, Reddy D, Negi PS. Development and validation of high performance liquid chromatographic method for the simultaneous determination of ceftazidime and sulbactam in spiked plasma and combined dosage form-Zydotam. American J applied Sci. 2009;6(10):1781-1786.
11. International conference on harmonization, (ICH) “Q2A: Text on Validation of analytical procedure,” Federal Register (notices), 1995; 65(40): 11260 11262.
12. International conference on harmonization, “Q2B- Validation of analytical Procedures: Methodology”, US Food and Drug Administration, Nov. 1996.

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