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# UPLC-MS/MS and Dushman reaction based spectrophotometric method for determination of Ceftazidime, an antibiotic, in medicinal formulation

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**ABSTRACT:**
Two analytical methods were developed, one utilizing sensitive UPLC-MS/MS method while other being the spectrophotometric method exploiting the Dushman reaction. Both methods were validated based on ICH guidelines and were successfully applied to quantitate the drug in marketed formulation. UPLC-MS/MS method responded linearly in the range of 1.6 $\mu gmL^{-1}$-6.4 $\mu gmL^{-1}$ while spectrophotometric procedure followed the Beer's law in the concentration range of 30-100 $\mu gmL^{-1}$. UPLC-MS/MS has been found to have a limit of quantitation of 0.97 $\mu gmL^{-1}$ while spectrophotometric method was found to quantitate 14.00 $\mu gmL^{-1}$ correctly with high precision. Both the methods showed an excellent recovery of 99.36%-100.91% (% RSD 1.05-1.73) and 98.64%-99.73% (%RSD 0.67-1.68) for UPLC-MS/MS and spectrophotometric, respectively.

**Keywords:** Ceftazidime; UPLC-MS/MS, method development; spectrophotometry.

**Practical Application:** Analysis of antibiotics in medicinal formulation.

## 1 Introduction

Ceftazidime, a third-generation cephalosporin with greater activity against Gram-negative bacteria is chemically recognized as (6R, 7R, Z)-7-(2-(2-aminothiazol-4-yl)-2-(2-carboxypropan-2-yloxyimino) acetamido)-8-oxo-3-(pyridinium-1-ylmethyl)-5-thia-1-aza-bicyclo [4.2.0] oct-2-ene-2-carboxylate.

Like other third generation cephalosporins, it is stable enough to resist inactivation by hydrolysis via b-lactamases produced by the gram +ve and gram –ve bacteria. The uniqueness of ceftazidime in its class of cephalosporin is its activity against the *Pseudomonas species* (Otani et al., 2018). Ceftazidime is generally prescribed for serious infections of the respiratory tract, soft tissues, abdominal viscera and infections of bones and joints (Abounassif et al., 1990). Cystic fibrosis and meningitis are the other conditions where ceftazidime treatment is recommended (Gozzard et al., 1982). Another import use of the drug includes its all-important usage in diabetic foot syndrome. Owing to its importance, world health organization has classified ceftazidime in the list of essential medicines. The list contains important drugs which is needed for basic health care. Chemically, $C=N-O-CH_3$ functional group is present in ceftazidime. As reported the human body practically cannot metabolize ceftazidime, thus 90-96% of ceftazidime is excreted in unaltered form (Tůma et al., 2016).

Ceftazidime is administered either intravenously (IV) or intramuscularly (IM), the dose and the frequency of administration depends largely upon the type and severity of the infection. However, it is used with caution in those with kidney impairment and the elderly (Abounassif et al., 1990). It has been reported that ceftazidime for injection can be reconstituted in sterile water however, for injections at certain cases, one such is veterinary use is prepared in 0.5% or 1% Lidocaine hydrochloride in water for injection (Papich, 2016).

Developments of analytical method for the determination of antibiotic concentrations in different matrices aims at offering a reliable, simple, faster or cheaper method which could be adopted in routine laboratories analysis, especially in countries with limited resources. To this extent, researchers in this field regularly offer new or improved methods, which may have some advantages over the earlier reported methods. It is critical that the concentration of antibiotic in question to correctly measure and to ensure that the therapeutic concentration has been reached. Moreover, very high concentrations may cause systemic toxicity (such as, high concentration of ceftazidime causes bone marrow depression and increase the liver enzymes level). Low concentrations on the other hand may allow the development of drug resistance by encouraging the bacteria to mutate or alter its metabolic pathway. Hence, it is not surprising that the measurement of ceftazidime level has been subjected to different analytical techniques ranging from the use of low cost instruments to highly sophisticated and ultrasensitive techniques. These reported techniques include, IR spectrometry (Moreno & Salgado, 2012a) and spectrophotometry (Salem &

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Samir, 2018; Arun et al., 2010; Krishna et al., 2013; Moreno & Salgado, 2008a; Mahramyari et al., 2014; Patel et al., 2011; Devkhile & Shaikh, 2011; Mohammed et al., 2019), HPLC (Moreno & Salgado, 2008b; Moreno & Salgado, 2012b; Hassouna & Mohamed, 2020; Siddiqui et al., 2009; Nanda & Shelke, 2013) and several other electoanalytical methods (Torkashvand et al., 2016; Hu et al., 2016; Shahrokhian et al., 2014; El-Maali, 2000). In the current communication, two different methods are developed which reliably measures ceftazidime concentration, methods include the spectrophotometry and the UPLC-MS/MS technique. Both the analytical methods are sensitive, rapid and require no sample pre-treatment procedures. One of the methods is based on the ionization of ceftazidime in positive mode and subsequently determination by UPLC-MS/MS (Method A), while the other exploits the Dushman reaction based spectrophotometric technique (Method B) to evaluate the ceftazidime content in pharmaceutical formulations.

## 2 Materials and method

### *2.1 Materials*

Ceftazidime was procured form Sigma, USA, and potassium iodide and potassium iodate was obtained from BDH chemicals, Poole England. Avonchem, Cheshire, UK manufactured ethanol was used for the UPLC-MS/MS studies, whereas, Milli Q purified water was used throughout the experiment. The Pharmaceutical formulation used was manufactured by cadila pharmaceuticals.

### *2.2 Equipment and experimental conditions*

The methods described in this communication were based on simple Spectrophotometer and advanced hyphenated UPLC-MS/MS system. The spectrophotometric measurements were carried out using Thermo manufactured, Evolution 300 UV-visible spectrophotometer; quartz cells were used as sample holder and the reaction were measured at 25 °C, and the yellow colored complex of ceftazidime-iodide-iodate was measured at 352 nm. The UPLC-MS/MS studies on ceftazidime determination were performed on water's manufactured Acquity ultra-performance liquid chromatography system combined with MS detector. MassLynx software was used for the data evaluation for the standard and the test samples. UPLC-MS/MS separations were performed on 100 mm × 2.1 mm UPLC BEH C18 column with particle size of 1.7 μm. Tuning conditions for mass detection were; capillary voltage-3.5kV, cone voltage- 20V, source temperature-120 °C, desolvation temperature-300 °C, desolvation gas flow-600 $Lh^{-1}$, cone gas flow-60 $Lh^{-1}$.

Standard stock solution: For Spectrophotometric determination of ceftazidime, $1.0 \times 10^{-2}$ M potassium iodide (KI), $1.82 \times 10^{-2}$ M potassium iodate ($KIO_3$) and $9.14 \times 10^{-4}$ M ceftazidime (25 mg in 50 mL) were prepared and were diluted as per the requirements. For the UPLC-MS/MS studies $1.83 \times 10^{-4}$ M (10 mg in 100 mL) stock solution was prepared. From the stock solution, 30-100 $\mu g mL^{-1}$ was prepared for spectrophotometric measurement and 1.6 to 6.4 $\mu g mL^{-1}$ was prepared for UPLC-MS/MS studies.

### *2.3 Experimental procedures*

*Method A*

From the 0.1 mg $mL^{-1}$ standard stock solution seven different ceftazidime concentrations with a lower limit of 1.6 μL $mL^{-1}$ and upper limit of 6.4 μL $mL^{-1}$ were prepared and individual sample was place in the UPLC-MS/MS system for analysis. The chromatographic separation was attained using C-18 column. Ethanol and water in a ratio of 10:90 was used as mobile phase which flew through the column at a rate of 0.2 mL per minutes. Five micro liters of the sample volume was injected throughout the experimental process. The chromatographic run time was 1 minutes and 0.75 minutes was taken by the ceftazidime to pass through the detector. Standard chromatogram of ceftazidime is mentioned in Figure 1.

*Method B*

From the standard stock solution, different concentrations of ceftazidime ranging from 30-100 μg $mL^{-1}$ were collected in a series of 5 mL standard volumetric flask. To each of the standard flask, 0.8 mL of $1.82 \times 10^{-3}$ M $KIO_3$ was added followed by 1.0 mL of $1.0 \times 10^{-2}$ M KI. Both the solutions were properly mixed and then diluted adding milli-Q water upto the mark. After mixing of the drug and the two reagents a yellow colored product was formed, absorbance of which was recorded at 352 nm. Following the absorbance measurement, a calibration graph was prepared by plotting the absorbance *versus* the concentration of ceftazidime. A regression equation was derived from plot was used for calculating the assay of drug. Additionally, calibration plot can also be used to evaluate the amount of ceftazidime drug.

## 3 Results and discussion

### *3.1 Method A*

(LC-MS/MS): Among the many important aspect of the chromatographic separation of an analyte is good sensitivity and symmetric peak. This could be achieved by optimizing the

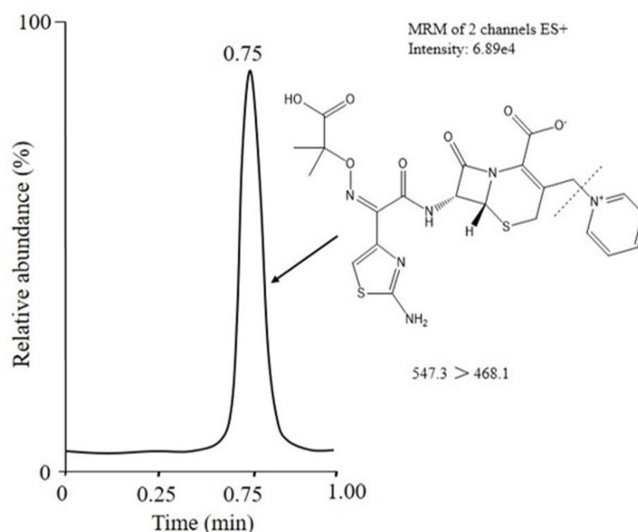



**Figure 1**. Standard chromatogram of ceftazidime.

chromatographic condition. The ionization and the estimation of the target analyte also depend on the solvent used during the analysis. In current analysis ethanol and water (10:90) was used as the mobile phase where the former acts as the organic modifier. With ethanol water combination as mobile phase excellent sensitivity and good peak shape was obtained, additionally, ethanol was opted as a part of the mobile phase as a green solvent with an intention to minimize the involvement of the toxic chemicals during the study. During the optimization of the mobile phase conditions, it was observed that the best peak in terms of shape and sensitivity was observed when the contribution of the organic modifier was 10% of the mobile phase. Increasing the contribution of the ethanol in the mobile phase led to the deformation of the peak and the sensitivity too. It has been reported that ethanol shares same characteristics to that of methanol and acetonitrile for reverse phase liquid chromatographic application. However, the viscosity of the ethanol water solution is higher than the two solvents which require a little more pressure. In case of UPLC-MS the instrument works on the pressure range of 6000-15,000 psi range as compared to the conventional chromatography which operates at 2000-4000psi. Thus UPLC-MS instrumentation has wide range of pressure range that can sustain elevated pressure from ethanol water combination additionally, it offers environmental friendly solvent as mobile phase (Destandau & Lesellier, 2008).

To get the maximum abundance the optimization of the MS parameters is equally significant which was achieved by infusing 0.5 µgmL$^{-1}$ ceftazidime solution into the into the MS setup. As per the obtained ESI - Q1 full ion spectra it was observed that there was insignificant signal intensities were obtained in the negative ionization mode whereas in the positive mode ceftazidime displayed an excellent signal intensity and generated precursor $[M+H]^+$ ions at 547 (*m/z*). Post ionization selection the optimization of the capillary and cone voltage was optimized and set at 3.5 kV and 20 V, respectively. The stable and predominant product ions were generated by optimizing the collision energy and collision gas flow and finally setting them to 20 eV and 0.10 mL/min respectively. At optimal condition the protonated parent was identified at *m/z* 547.27 and while the two most abundant daughter ions were characterized by *m/z* 468.09 and *m/z* 167.19. Considering most abundant molecular ions MRM transition of *m/z,* 547.2 → 468.1 was selected for the quantitation studies. The MRM transition of ceftazidime and its fragmentation pattern has been shown in Figure 2.

### 3.2 Method B

Considering the case of method B, where iodate induced oxidation was used for the quantitative evaluation of ceftazidime. Feigl in his book suggested that in presence of iodide and iodate, the water soluble acid compounds tend to liberate iodine as per the following reaction sequence as mentioned in equation 1 (Feigl, 1960).

$$I^- + IO_3^- + 6H^+ \rightarrow 3H_2O + 3I_2 \quad (1)$$

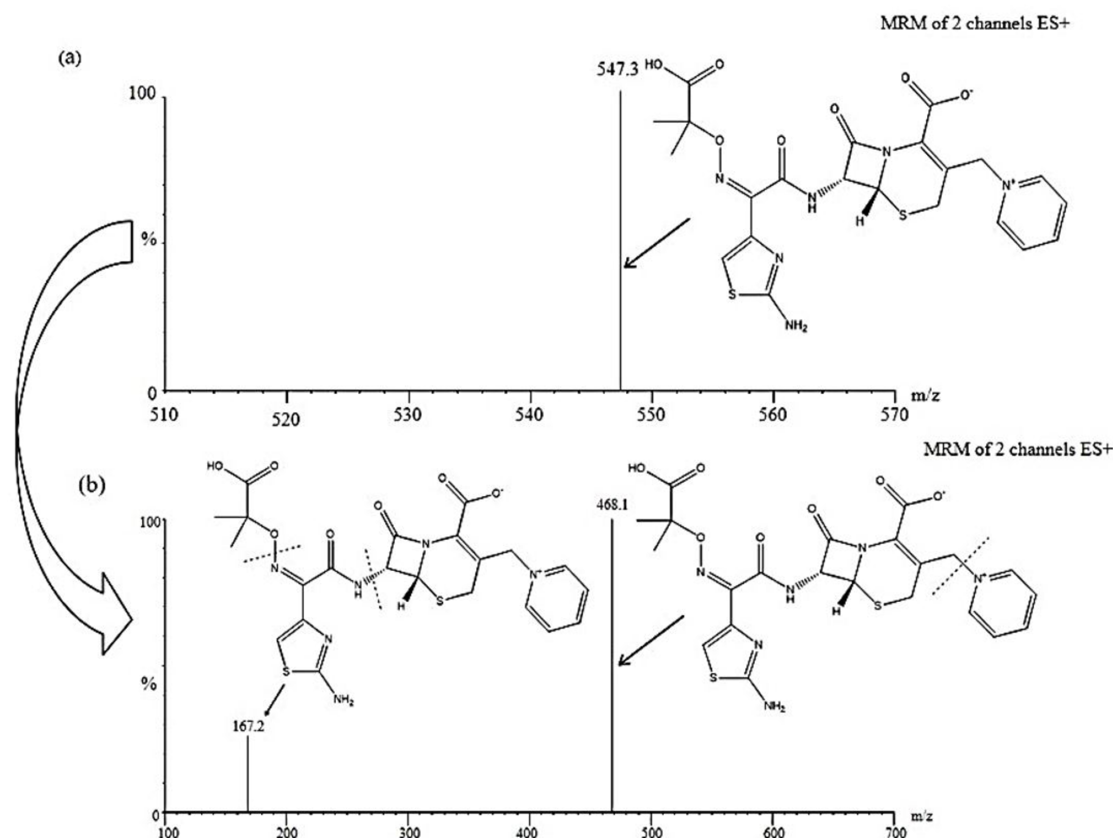



**Figure 2**. MRM spectra and fragmentation pattern of ceftazidime.

The same reaction has elaborately been discussed (Xie et al.,1999), the same reagent and the reaction mechanism was previously followed for the determination of irbesartan (Rahman et al., 2006) amoxicillin (Qarah et al., 2020) and ramiprilin (Rahman et al., 2005).

In the current investigation both the reagent and the drug are color less compounds, upon adding ceftazidime in the mixture of the iodate and iodide, the solution turn yellow which forms the basis of the quantitation of the target drug. Optimization experiments were conducted to get the exact amount of the iodide and iodate required to get the complete reaction. Effect of $KIO_3$ was checked with increasing the concentration from $1.83 \times 10^{-05}$ M, it was found that the absorbance increased up to $1.82 \times 10^{-4}$ M after that remained as such up to $2.92 \times 10^{-4}$ M (Figure 3).

Similar experiments were conducted to check the effect of KI which was found to increase from $2.0 \times 10^{-04}$ M to $1.6 \times 10^{-3}$ M after this concentration it remained constant up to $2.4 \times 10^{-3}$ M (Figure 4). After the optimization experiments spectral studies were conducted involving the blank, the drug and the reagent-drug complex. From the spectral studies (Figure 5), it can be seen that the ceftazidime shows absorption maxima at 260 nm whereas the blank solution displays two peaks 230 and 270 nm. Reaction starts upon addition of the drug to the blank solution and the solution turns yellow and there is new characteristics band appearing at 352 nm. This 352 band can possibly be attributed to the formation of the triiodide ion.

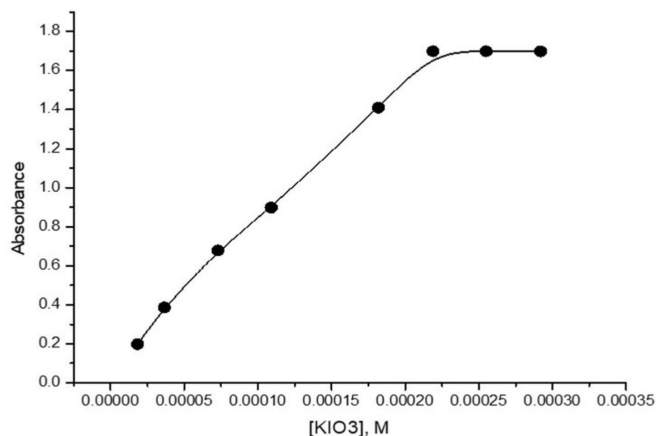



**Figure 3**. Effect of $KIO_3$ on the color development.

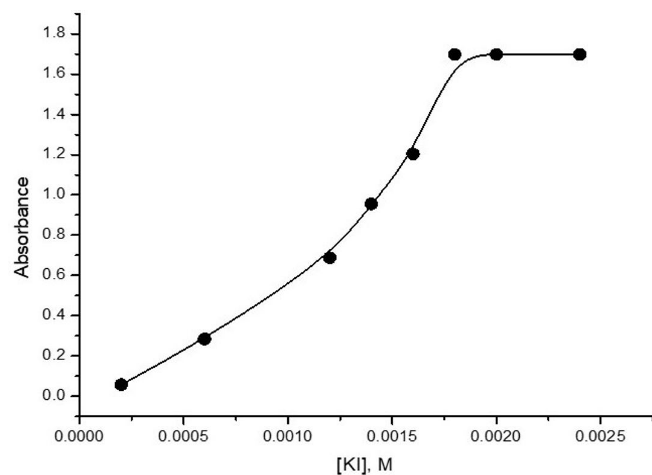



**Figure 4**. Effect of KI on the color development.

The formation of yellow colored compound is due oxidation of iodide by iodate in presence of the acid compound (ceftazidime) to form iodine, which further react with iodide and form triiodide ion as final products. The complete reaction mechanism is shown in Scheme 1.

### *3.3 Method validation*

Proper validation studies have become an indispensable part of successful method development and drug analysis. The term "validation of method" refers to the experimental setup to ascertain the method provide accurate result with significant precision. In our current study both the developed methods were tested and made sure that they pass the validation criteria. The experiments used for evaluation of the method's suitability were linearity, accuracy, precision, detection limit, robustness and stability studies. The regressional characteristics of the two methods are mentioned in Table 1.

### *3.4 Calibration plot*

The acquisition of the calibration plot was performed by plotting peak area against the concentration of the ceftazidime. In UPLC-MS/MS procedure, the seven points calibration plot ranging between 1.6 $\mu gmL^{-1}$-6.4 $\mu gmL^{-1}$ yielded a correlation coefficient of 0.990. The linear regression equation obtained from the plot was found to be $A = -104.53 + 308.88 \times C$. The spectrophotometric procedure was tested for its linear range at calibration point of 30, 50, 60, 70, 80, 90 and 100 $\mu gmL^{-1}$, which produced a correlation coefficient of 0.9986 and resulting linear regression equation was found to be $A = -0.155 + 0.0157 \times C$.

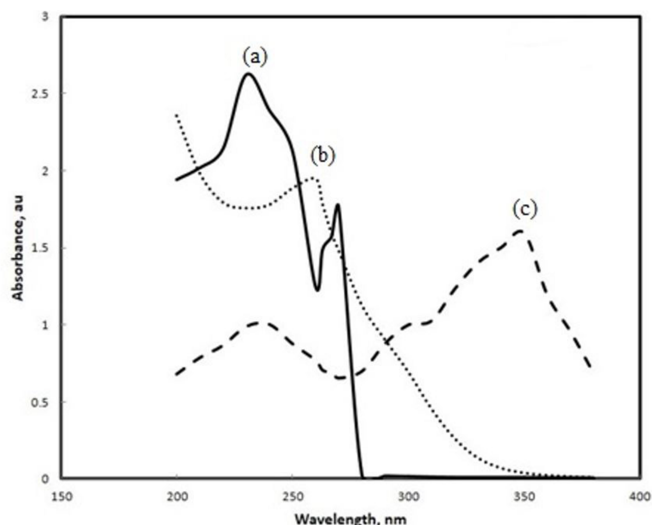
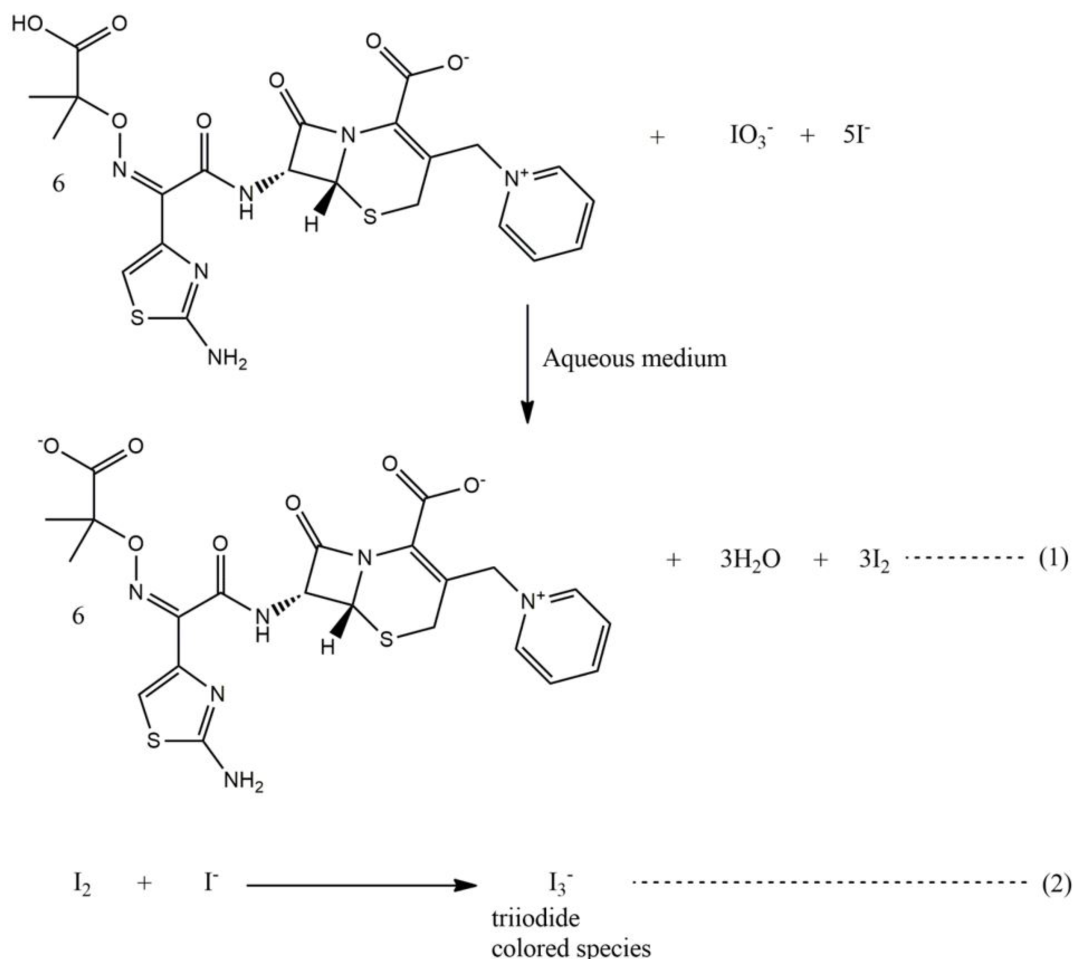



**Figure 5**. Absorption spectra (a) $2.92 \times 10^{-4}$ M $KIO_3$ + $2.0 \times 10^{-3}$ M KI; (b) $7.31 \times 10^{-5}$ M Ceftazidime; (c) $2.92 \times 10^{-4}$ M $KIO_3$ + $2.0 \times 10^{-3}$ M KI + $1.83 \times 10^{-4}$ M Ceftazidime.




**Scheme 1**. Schematic representation of the reaction sequence for the color development between mixture of KIO3 and KI and Ceftazidime.

*3.5 Accuracy and precision*

Two are closely related term where the former addresses the closeness of the agreement between the obtained value and the real value whereas as the later represent the closeness of agreement between the series of data. Although both are different terminologies yet they are closely related to each other and are of low significance without each other in concentration points i.e. 2.4, 4.8 and 6.4 µgmL$^{-1}$ for UPLC-MS studies and 30, 60 and 90 µgmL$^{-1}$. The precision studies were performed taking into account the repeatability and data interpretation. The accuracy and precision studies were performed at three intermediate precision. Repeatability studies were performed over short time duration under similar experimental environments. Whereas the intermediate precision was performed varying the days, analyst and the sample preparation glassware. Percent recovery was used to show the accuracy of the method while standard deviation was the tool to show the precision of the method. In ours study in UPLC-MS/MS method % recovery varied from 99.72-101.06 while the % RSD range was 0.45-1.91 while in spectrophotometric method accuracy in terms recovery was found in range of 98.96%-99.97% while precision in terms of % RSD lies in between 0.65-1.74. The compilation of the accuracy and precision studies are stated in Table 2.

**Table 1**. Regressional and UPLC-MS characteristics for determination of ceftazidime.

| Analytical Parameters | Spectrophotometry | UPLC-MS/MS |
|---|---|---|
| $\lambda_{max}$ / Ionization mode | 352 | Positive |
| Linear dynamic range | 30-100 | 1.6-6.4 |
| Linear regression equation | A = -0.1554+ 0.0157*C | A = -155.65 + 330.56 *C |
| $S_0^*$ | 0.0220 | 29.27 |
| Intercept (a) | -0.1554 | -155.65 |
| Slope (b) | 0.0157 | 330.56 |
| Correlation coefficient (r) | 0.9986 | 0.9989 |
| Detection limit | 4.62 | 0.32 |
| Quantitation limit | 14.0 | 0.969 |

*$S_0$ – Standard deviation of the calibration curve.

***3.6 Detection and quantitation limits***

To get information about the lowest amount of the ceftazidime that can be detected and quantitate, both signal and the noise were determined by injecting the sample into the UPLC-MS/MS system. 6 blank samples followed by 6 replicates of the target analyte at 0.5 µgmL$^{-1}$ signal to noise ratio was established and LOD was calculated

**Table 2**. Accuracy and precision studies for the determination of ceftazidime by LC-MS/MS and Spectrophotometry.

| Precision | Theoretical | Spectrophotometry | | | LC-MS/MS | | |
|---|---|---|---|---|---|---|---|
| | | 30 | 60 | 90 | 2.4 | 4.8 | 6.4 |
| Intra-day | Nominal ± SD | 29.81 ± 0.42 | 59.74 ± 0.39 | 89.88 ± 0.65 | 2.39 ± 0.02 | 4.76 ± 0.07 | 6.40 ± 0.09 |
| | Recovery ± RSD | 99.35 ± 1.40 | 99.57 ± 0.65 | 99.86 ± 0.73 | 99.94 ± 0.91 | 99.20 ± 0.03 | 100.02 ± 1.42 |
| Inter-day | Nominal ± SD | 29.69 ± 0.52 | 59.98 ± 0.46 | 89.96 ± 0.99 | 2.41 ± 0.04 | 4.81 ± 0.07 | 6.39 ± 0.12 |
| | Recovery ± RSD | 98.96 ± 1.74 | 99.97 ± 0.77 | 99.96 ± 1.09 | 100.34 ± 1.79 | 100.23 ± 1.62 | 99.92 ± 1.92 |

**Table 3**. Standard addition method for the recovery studies of ceftazidime by Spectrophotometry and UPLC-MS/MS.

| Formulation | Spectrophotometry | | | | | LC-MS/MS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Theoretical | Spiked | Nominal | RSD | Recovery | Theoretical | Spiked | Nominal | RSD | Recovery |
| Bectozid 1gm | 30 | 30 | 59.19 | 0.80 | 98.64 | 1.6 | 1.6 | 3.18 | 1.05 | 99.36 |
| | 30 | 40 | 69.81 | 0.67 | 99.73 | 1.6 | 2.4 | 4.00 | 1.73 | 100.09 |
| | 30 | 50 | 79.74 | 1.68 | 99.67 | 1.6 | 3.2 | 4.78 | 1.21 | 99.50 |
| | 30 | 70 | 98.91 | 1.33 | 98.91 | 1.6 | 4.8 | 6.46 | 1.40 | 100.91 |

as 3 times S/N and LOD 10 times of the same. For UPLC-MS/MS method LOD and LOQ was found to be 0.32 and 0.969 $\mu gmL^{-1}$.

For the spectrophotometric method LOD and LOQ was calculated using the linear regression equation where the slope and the standard deviation of the calibration line was used to evaluate the same using the equation LOD = $3.3\times S_0/b$ and LOQ = $10\times S_0/b$. In spectrophotometric method LOD and LOQ was found to be 4.62 $\mu gmL^{-1}$ and 14.00 $\mu gmL^{-1}$, respectively.

### *3.7 Stability studies*

The stability of the ceftazidime solution was check at two storage condition level i.e. at room temperature (25 °C) and at refrigerated condition (2-8 °C). At both the storage condition the sample was checked for its assay at 0h, 2h, 4h, 8h, 12h, 18h and 24 h. At the end of 24 h the assay at room temperature was found to be 94.3% while at the refrigerated condition the same was found to be 98.5%.

### *3.8 Recovery studies*

Instrument system components and instruments itself are sometime involved in the uncontrolled random errors, to control such error in the developed analytical procedure standard addition technique is employed where the pure drug was added to the preanalyzed formulation concentration at 4 concentration points, subsequently the recovery was calculated. In UPLC- MS/MS method the recovery ranged from 99.36%-100.91% (% RSD 1.05-1.73), while the spectrophotometric procedure yielded an assay of 98.64%-99.73% (%RSD 0.67-1.68). Detailed recovery studies are mention in Table 3.

## 4 Application of the proposed method

The developed were applied to check the amount drug in pharmaceutical formulation, Bectozid 1 gm. The amount of drug was found to comply with the label claim.

## 5 Conclusions

The purpose of this work was to develop new analytical methods based on either the low cost technology that could be adopted by smaller laboratories with limited resources or a highly sensitive UPLC-MS/MS method which could be used by advanced laboratories and research institutions. We have validated both these methods as per the international guidelines of international conference on harmonization (ICH) to confirm its authenticity for further use. Both the methods were successfully applied to quantitate the drug in marketed formulation. Both the methods are less time consuming and required no pretreatment prior to analysis. Between the two developed methods, UPLC-MS/MS method responded linearly in the range of 1.6-6.4 $\mu gmL^{-1}$, while spectrophotometric procedure followed the Beer's law in the range of 30-100 $\mu gmL^{-1}$. Considering limit of quantitation parameter UPLC-MS/MS has been found to have a minimum quantitation of 0.969 $\mu gmL^{-1}$ while spectrophotometric method was found to quantitate 14.00 $\mu gmL^{-1}$ correctly with high precision. Both the methods, UPLC-MS/MS and Spectrophotometry, showed an excellent recovery of 99.36%-100.91% (% RSD 1.05-1.73) and 98.64%-99.73% (%RSD 0.67-1.68). respectively. Furthermore, a faster and technically less demanding method to determine ceftazidime concentration in pharmaceuticals can ensure that therapeutic levels have been achieved thus avoiding drug toxicity or a lesser than required dose. It is known that drug resistance is caused by indiscriminate use of antibiotics e.g. when used in inappropriate conditions or when given in low dosage. Thus, the technique described by us has the potential to reduce antibiotic resistance by quickly warning the clinician that the antibiotic levels are inappropriately low.

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