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Original Article

RP-HPLC Forced Degradation Studies of Aztreonam in Pharmaceutical Dosage Form

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ABSTRACT

Introduction: Aztreonam belongs to the monobactam class of antibiotic used to treat infectious diseases produced by gram-negative bacteria. This work presents the development of LC methods for the estimation of Titled drug in pharmaceutical dosage form and forced degradation studies on four different stress conditions. **Method:** A waters HPLC Inspire (4.6 x 250mm, 5µm) in isocratic mode, with mobile phase containing buffer: Acetonitrile (40:60 %v/v) pH 3 adjusted with orthophosphoric acid were used. The flow rate was 1ml/min and linearity range was established at 5- 25 µg/ml. **Conclusion:** Degradation studies disclose method abilities on various stress conditions. Forced degradation results can be used for the development of stable dosage form and for the designing of proper storage requirement. The proposed method is accurate, precise, specific and rapid for the estimation of aztreonam injection.

Key words: Aztreonam, RP- HPLC, Forced degradation studies, Method development.

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INTRODUCTION

Aztreonam[1,2] is a synthetic monobactam bactericidal antibiotic originally isolated from chromo bacterium violaceum. It is a white crystalline powder. Aztreonam is chemically (z)-2- [(2- amino - 4-thiazolyl) [[(2S - 3S) - 2- methyl- 4- oxo -1- sulfo -3-azetidinyl] carbomoyl] methylene] amino] oxy] -2- methyl propionic acid, which is used in the treatment of life threatening infections with susceptible gram-negative aerobic organisms, especially Pseudomonas aeruginosa. Aztreonam acts by inhibiting bacterial cell wall peptidoglycan synthesis. Literature reviews[3-11] revealed, no stability indicating method of estimation for aztreonam by high performance liquid chromatography[12-14] has been reported so far except in biological fluids.

MATERIALS AND METHOD

Reagents and chemicals

Aztreonam was obtained from APL research lab Hyderabad. Methanol, water (LC-grade) and orthophosphoric acid were obtained from Merck. LC grade Acetonitrile were purchased from Molychem. Analytical grade sodium hydroxide (NaOH), Hydrochloric acid (HCl), were obtained from Fischer scientific. Hydrogen peroxide (H_2O_2) and 0.22 µm membrane filter were obtained from Sigma–Aldrich. Formulation was procured from the local market having strength of 500 mg/vial. All chemicals were of analytical or LC-grade. All the measurements were made using Waters HPLC. All the solutions were freshly prepared by using HPLC grade solvents.

Optimized chromatographic conditions

Instrument used: Waters HPLC with auto sampler and 2487 UV detector with Empower 2 software.

Temperature:	Ambient
Column:	Inspire (4.6 x 250mm, 5µm)
Mobile Phase:	buffer pH 3: Acetonitrile 40:60
Flow rate:	1 ml per min
Wavelength:	255 nm
Injection volume:	20 µl

Stock and working solution

10 mg of aztreonam was taken in a 100 ml standard flask and the volume was made up to 100 ml with water to get a concentration of 100 µg/ml of aztreonam. From the above solution, further dilutions were made to get concentrations from 5-25 µg/ml.

Preparation of sample solution

The whole content of the vial was transferred to a 100 ml standard flask and dissolved in 10 ml of water for injection. Then the volume was made up to 100 ml using water (HPLC grade). From this 1 ml was taken and diluted to 10ml. From the above solution 2 ml was taken and diluted to 10 ml (100 µg/ml). The above solution was further diluted to get concentrations ranging from 5-25 µg/ml. The amount of drug present in the vial was calculated as follows.

$$\text{Amount of drug present} = \frac{\text{Concentration X dilution factor}}{\text{Volume taken}}$$

Degradation Studies

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing should be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on aztreonam using the proposed method.

Preparation of stock

Accurately weighed and transferred 10 mg of aztreonam working standard into a 10 ml clean dry volumetric flask, added about 7 mL of Diluent and sonicated to dissolve it completely and made volume up to the

mark with the same solvent.

Hydrolytic degradation under acidic condition

Pipetted out 0.75 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1 N NaOH and made up to 10ml with diluent. Filtered the solution with 0.22 microns syringe filters and placed in vials.

Hydrolytic degradation under alkaline condition

Pipetted out 0.75 ml of above solution into a 10ml volumetric flask and 3ml of 0.1N NaOH was added in 10 ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 6 h. Then neutralized with 0.1 N HCl and made up to 10ml with diluent. Filtered the solution with 0.22 microns syringe filters and placed in vials.

Thermal induced degradation

Aztreonam sample was taken in petridish and kept in Hot air oven at 110ºC for 24 hrs. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

Oxidative degradation

Pipetted out 0.75 ml of above stock solution into a 10 ml volumetric flask and 1 ml of 3% w/v of hydrogen peroxide was added and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filtered the solution with 0.45 microns syringe filters and placed in vials.

Photo degradation

Pipetted out 0.75 ml of above stock solution into a 10 ml volumetric flask and exposed to sunlight for 24 hrs and the volume was made up to the mark with diluent. Filtered the solution with 0.45 microns syringe filters and placed in vials.

METHOD VALIDATION

Accuracy

To study the reliability and accuracy of the method, recovery experiments were carried out. To the formulation equivalent to 10 µg/ml, standard aztreonam was added at the level of 50 % and 100 %. These were further diluted by the procedure followed in the estimation of formulation. The concentration of the drug present in the resulting sample solution was determined. The recovery procedure was repeated 3 times and the percentage recovery was calculated using the formula,

Where,

a = the amount of drug sample

b = the amount of drug sample + standard drug

c = the amount of standard drug added

Precision

$$\% \text{ Recovery} = \frac{b - a \ X\ 100}{c}$$

Method precision: A sample solution of aztreonam was prepared. A sample containing 15 µg/ml of Aztreonam was injected 3 times.

Intermediate/ruggdness: It was carried out by injecting a concentration (15 µg/ml) of the sample solution with different 'make' column and standard deviation was calculated.

Linearity and Range

Calibration curve was well established in the range of 5-25 µg/ml with correlation co-efficient value 0.9998

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ of the developed method were determined by injecting progressively low concentration of the standard solution under optimized chromatographic conditions.

The LOD and LOQ of Aztreonam were found to be 20 ng/ml and 5 µg/ml respectively.

Stability

In the present study, mobile phase, standard and sample solutions were subjected to 3 days stability studies. The stabilities of these solutions were studied by performing the experiment, looking for change in retention time, resolution, tailing of the peaks etc when compared to the pattern of the chromatogram of freshly prepared solution. The solution stored under room temperature was stable for 2 hrs and under refrigeration for 24 hrs.

System suitability studies

The system suitability studies were carried out as specified in USP. These parameters include column efficiency, resolution, peak asymmetry factor, capacity factor, peak tailing factor and percentage co-efficient of variation for peak area or height of repetitive injection. Although USP requires only two of these criteria for method validation, parameters like column efficiency (N), resolution (Rs), capacity factor (K), selectivity factor (α) and peak asymmetry factor (As) were calculated in this study.

RESULTS AND DISCUSSION

Optimization of Separation conditions[15-17]

Different ionic strengths such as 10, 25 mM solutions of potassium dihydrogen phosphate, adjusted to pH 2.6 were initially employed for the separation of aztreonam in the ratio of 20:80 (methanol: buffer). In all the cases aztreonam showed split peak with tailing, but in the case of buffer pH 3: Acetonitrile 40:60, a symmetrical peak with good separation was achieved.

Validation of the method

Validation studies[18-19] were carried out on different parameters as per ICH guideline such as recovery study, method precision and intermediate precision, range, LOD and LOQ, System suitability study. Linearity was established at five different concentrations with good correlation coefficient. Accuracy or recovery was conducted at different levels. Precision studies were carried out using the same optimized conditions and RSD[20-21] was less than 2 %. LOD and LOQ indicate, the method was highly sensitive and fast. The method was found highly specific since there was no interference from the excipients of the formulations. Analytical solution was found stable up to 24 hrs on refrigeration (Table 1).

Forced degradation studies

Different stress conditions[22,23] were used in the study. In case of hydrolytic stress degradation under acidic 0.1N hydrochloric acid was used as reagent whereas in alkaline condition 0.1N sodium hydroxide was incorporated. Drug was refluxed with acid, alkali and water for about 6 hrs at 60^0C targeting 5-20% degradation. In oxidative degradation studies

Table 1: Validation of developed RP-HPLC method

Recovery *	
100 % level	102.3± 0.19
Precision*	
Method	144886.7±273.2697
Intermediate/ruggdness	145772.6±402.134
Linearity Range	5 –25µg/ml
LOQ	5 µg/ml
LOD	20 ng/ml
System suitability parameters	
USP Plate count	9097.50
USP Tailing factor	0.95333
Stability	
Room Temperature	2 h
Refrigeration	1 day

Recovery-Average mean three determinations in each level, Average mean of six determinations for precision.

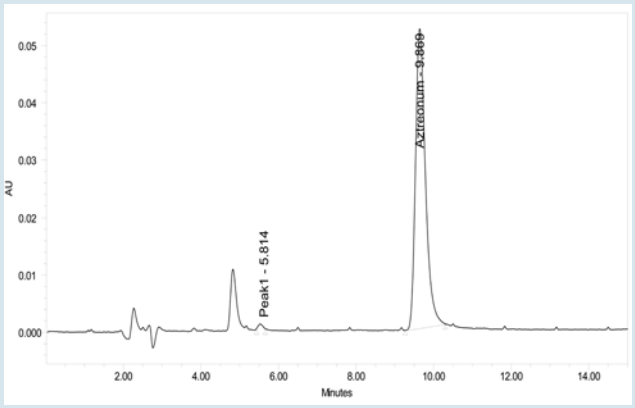

Figure 1: Acid degradation of Aztreonam.

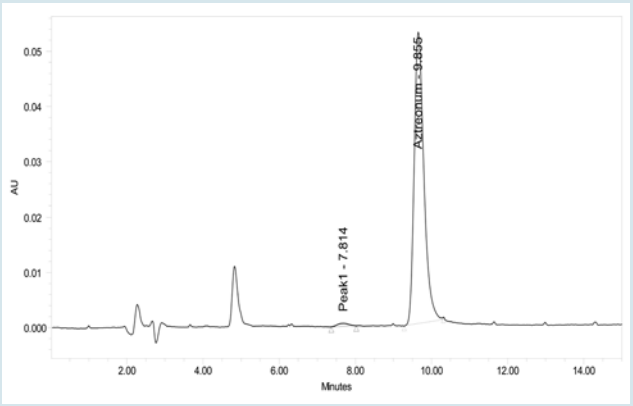

Figure 2: Alkaline degradation of Aztreonam.

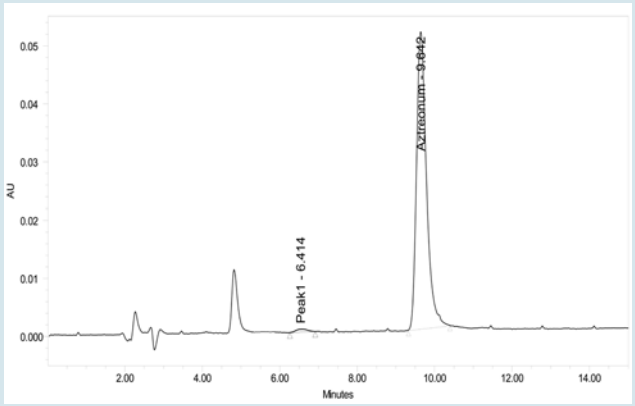

Figure 3: Peroxide degradation of Aztreonam.

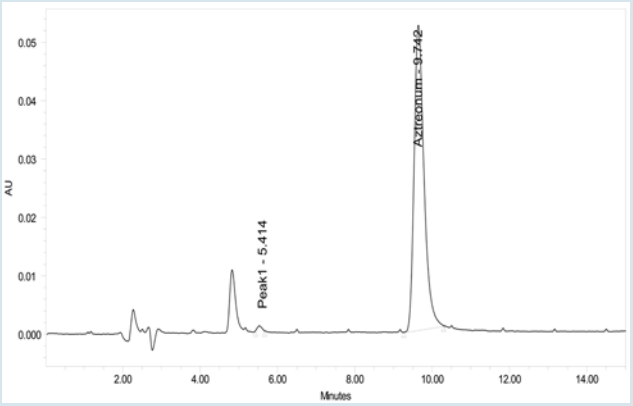

Figure 4: Thermal degradation of Aztreonam.

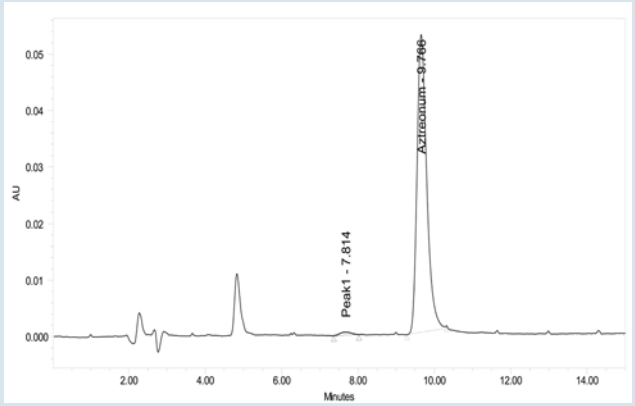

Figure 5: Photolytic degradation of Aztreonam.

Table 2: Forced degradation study

Sample Name	Aztreonam			
	Area	% Degraded	Purity angle	Purity threshold
Acid	136657	5.68	0.426	0.986
Base	141134	2.59	0.426	0.986
Peroxide	135151	6.72	0.426	0.964
Thermal	138744	4.24	0.426	0.986
Photo	137802	4.89	0.426	0.986

3% v/v hydrogen peroxide was used and kept at room temperature for 15 min. In thermal studies, conducted for 24 hrs at 110°C. In photolytic degradation, drug was exposed to sunlight for 24 hrs. Slightly high degradation was noted for peroxide stress condition than other conditions. Only one degradant peak was observed which did not interfere with the main peak. From the degradation studies, the peak angle of the drug was less than the purity threshold which indicates there was no merging of impurity peak with the analyte peak (Table 2 and Figure 1-5).

CONCLUSION

A Rapid, stable and sensitive stability indicating assay method was developed and validated**.** Extent of degradation increases with an increase in the duration of stress studies and the degradant peak was not interfering with main peak. It can be concluded that the developed method is stable for specified period of time in different stress conditions. This method can be extended for characterization of the degradants with integrated approach and they can be effectively applied for routine analysis in various drug testing departments.

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CONFLICT OF INTEREST

Nil

ABBREVIATION USED

RP-HPLC: Reverse-phase High-performance liquid chromatography; NaOH: sodium hydroxide; HCl: Hydrochloric acid; LC: Liquid chromatography.

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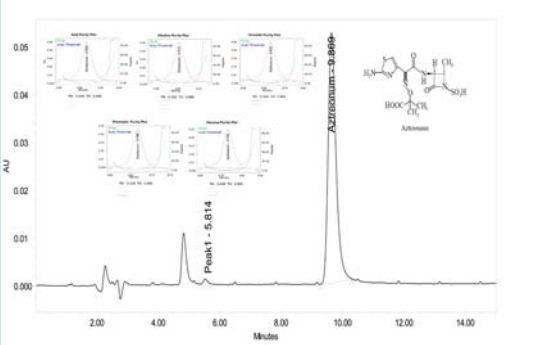
PICTORIAL ABSTRACT



SUMMARY

- This overlay contain peak purity plot and degradation chromatogram of titled drug
- This analytical technique was found to be simple,rapid, economical, accurate and precise and can be effectively applied for routine analysis of titled drug in pharmaceutical formulation

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