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VALIDATION FOR QUANTITATIVE DETERMINATION OF AZTREONAM IN SIMULATED LUNG FLUID BY UV SPECTROSCOPY METHOD

Sayani Bhattacharyya * and Bharani S. Sogali

Department of Pharmaceutics, Krupanidhi College of Pharmacy, Bangalore - 560035, Karnataka, India.

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Correspondence to Author:
Sayani Bhattacharyya

Assistant Professor,
Department of Pharmaceutics,
Krupanidhi College of Pharmacy,
Bangalore - 560035, Karnataka, India.

E-mail: sayanibh@gmail.com

ABSTRACT: Aztreonam, a synthetic beta-lactam antibiotic is widely used for the treatment of infections in lungs, meninges, bladder, *etc*. Its potential use in the treatment of pneumonia and cystic fibrosis has been established. Recently FDA has approved Cayston® for inhalation therapy of Aztreonam in lungs infection. Many studies have been reported on the quantitative determination of Aztreonam by UV spectrophotometric and HPLC method. The present study focuses on the quantitative spectrophotometric determination of Aztreonam in two simulated lungs fluid namely artificial lysosomal fluid (ALF) and gamble solution. The λ_{max} was found at a wavelength of 293 nm for both the solutions. Further, the method developed was validated for its linearity, precision by interday and within day study, accuracy, specificity, robustness and determination of limit of quantification and limit of detection in both the solutions. The linearity demonstrated a correlation coefficient of 0.9995 and 0.9999 in ALF and gamble solution respectively. The LOD was found to be 0.38 µg/ml and 0.18 µg/ml for ALF and gamble solution respectively. The LOQ was found to be 1.15 µg/ml and 0.53 µg/ml for ALF and Gamble solution respectively. The proposed method was found to be simple, rapid, accurate, precise, and specific for the determination of Aztreonam in simulated lungs fluid.

INTRODUCTION: Aztreonam is a synthetic monobactam (monocyclic beta-lactam) antibiotic, originally isolated from *Chromobacterium violaceum*. It is used in gram-negative infections including *Pseudomonas aeruginosa*, especially used in the infection of the meninges, bladder, and kidneys. It is also the drug of choice for bacteremia, bone infection, intra-abdominal infection, kidney infections, pneumonia, pneumonia with cystic fibrosis, skin and urinary tract infection. Aztreonam preferentially binds to and inactivates penicillin-binding protein-3 (PBP-3), which is primarily involved in bacterial cell wall synthesis, thereby inhibiting bacterial cell wall integrity and leading to cell lysis and death.

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Aztreonam differs from other beta-lactam antibiotics because it is resistant to beta-lactamase hydrolysis, produced by most gram-negative and gram-positive pathogens; therefore it is usually used to treat infections caused by gram-negative aerobic microorganisms. The IUPAC name of Aztreonam is (2S, 3S)-3-{[(2Z)- 2- (2-Ammonio-1, 3-thiazol-4-yl) -2 -{[(2-carboxy-2 -propanyl) oxy] imino} acetyl] amino}-2-methyl- 4-oxo- 1-azetidinesulfonate. Aztreonam maintains its antimicrobial activity over a pH range of 6 to 8 *in-vitro*, as well as in the presence of human serum and under anaerobic conditions [1].

Review of literature revealed that several methods are existing for assay of Aztreonam using different techniques such as HPLC with UV detection, RP-HPLC, spectrophotometric and fluorometric [2, 3, 4]. Aztreonam has been recently marketed in inhalation form (Cayston®) for the treatment of pneumonia, and many types of research showed potential use of Aztreonam in inhalation form [5].

However, there is no simple and accurate method reported for the detection of Aztreonam in lung fluid (pH in the range of 5-6.5) by UV spectrophotometry. It is felt necessary to establish an accurate, simple, fast, and economic method for quantitative determination of Aztreonam in solutions of pH<6.0 and solutions which resemble lung fluid like artificial lysosomal fluid (ALF) and gamble solution for evaluation of inhalation formulations of Aztreonam.

MATERIALS AND METHODS: Aztreonam was procured as a gift sample from Fuan Pharmaceutical Group Chongqing, Bosen Pharmaceutical Co. Ltd., China. Chemicals, as required to make simulated lung fluids, were all analytical grade.

Preparation of Simulated Lungs Fluid: Simulated lung fluid (SLF) consists of lipid-rich lipoproteins mainly phosphatidylcholine with a high dipalmitoyl content. About 85-90% of the isolated surfactant of lungs fluid is lipid of which 95% is phosphoglycerols with cholesterol as the main neutral component.

TABLE 1: COMPOSITION OF SIMULATED LUNGS FLUID

Compositions	ALF (g/l)	Gambles solution (g/l)
Magnesium chloride	0.50	0.095
Sodium chloride	3.21	6.019
Potassium chloride	-	0.298
Di-sodium hydrogen phosphate	0.071	0.126
Sodium sulfate	0.039	0.063
Calcium chloride di-hydrate	0.128	0.368
Sodium acetate	-	0.574
Sodium bicarbonate	-	2.604
Sodium citrate di-hydrate	0.077	0.097
Sodium hydroxide	6.00	-
Citric acid	20.8	-
Glycine	0.059	-
Sodium tartrate di-hydrate	0.90	-
Sodium lactate	0.085	-
Sodium pyruvate	0.086	-
Water q.s.	1000 ml	1000 ml

Many proteins are also present. Presence of albumin, which might be a contaminant, includes four non-serum apoproteins. SLF has been used to evaluate human exposure to particulate matter from environmental emissions. Artificial lysosomal fluid (ALF) and gamble's solution are used to simulate different interstitial conditions in the lung. ALF is analogous to the fluid with which inhaled particles would come into contact after phagocytosis by alveolar and interstitial macrophages in the lung. Gamble's solution represents the interstitial fluid deep within the lung. Gamble's solution has a pH of 7.4, whereas ALF has a pH of 4.5 and has a much higher organic content than Gamble's solution [6, 7]. The composition of ALF and Gamble solution is presented in **Table 1**.

Calibration Procedure: A stock solution of Aztreonam in ALF and gamble solutions system was prepared separately at a concentration of 100µg/ml. It was further diluted to 100 ml to obtain a solution of concentration corresponding to 10µg/ml. The absorbance of the resulting solution was scanned in the UV spectrometer (UV-Vis spectrophotometer Tech comp 23.1) in the range 200-400 nm to determine the maximum wavelength (λ_{max}). The same method was followed for both the solutions to generate the calibration curves. A calibration curve was drawn at the λ_{max} by varying the concentration of the diluted stock solution from 2-12 µg/ml. ALF and gamble solutions were taken as blank for their respective dilutions. A graph was plotted with the absorbance and concentrations (2-12 µg/ml) at triplicate with the standard deviation.

Method Validation: The methods were validated as per the ICH guidelines on analytical process validation. The linearity, accuracy, precision, specificity, and robustness of the method were validated as per ICH guidelines [8].

Determination of Linearity: The calibration curves were obtained with six different concentrations of the standard solutions of Aztreonam (2-12 µg/ml) in ALF and gamble solution. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method [9].

Determination of Precision: The assay precision was carried out by repeatability study. Interday and within day repeatability were evaluated for both the solutions at three different time points. Six samples of Aztreonam (1-6 µg/ml) in ALF and gamble solution were analyzed at three different time points. The % RSD was calculated for both the methods [10, 11].

Determination of Accuracy: Accuracy of the method in different solvents system was determined by the recovery assay. The accuracy of an analytical method determines the closeness of

the test results obtained by the method to the true value. A known concentration of working standard was added to pre-analysed sample solution at three different levels, *i.e.* 50%, 100%, and 150%, absorbance was recorded, and % recovery was calculated. The recovery results were recorded and % RSD was calculated [12].

Determination of Specificity: Specificity allows determination of the compound in the presence of other excipients. So, a marketed product of Aztreonam, Azenam injection 500 mg / 10 ml from Aristo Pharmaceuticals Pvt. Ltd., India was taken, and the content was determined at different dilutions by spectrophotometrically. The content was determined in triplicate in ALF and Gamble solution spectrophotometrically at a dilution of 5µg/ml and verified with the labeled content. The average content and % RSD were calculated in each solution [13].

Determination of Robustness: The robustness of the spectrophotometric method was checked by allowing a small change in pH of the solutions (± 0.2 units) and wavelength (± 1 unit) and % RSD was found out.

Limit of Detection and Limit of Quantification: The limit of detection (LOD) and limit of quantification (LOQ) were determined according to ICH guidelines [14].

$$LOD = 3.3 * SE / A \quad (1)$$

$$LOQ = 10 * SE / A \quad (2)$$

SE = Standard error of Y intercept, A = Slope of the calibration curve.

RESULTS AND DISCUSSION: Aztreonam contains specific chromophores in the structure that absorb at a wavelength. This fact has been successfully employed for its quantitative determinations using UV spectroscopic method which is simple, rapid, sensitive, precise and cost-effective. The solutions of Aztreonam in ALF and gamble solutions showed maximum absorbance at a wavelength of about 293 nm as shown in **Fig. 1** and **Fig. 2**.

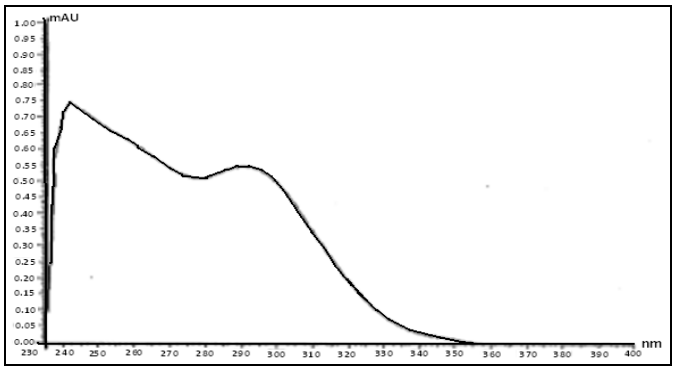

FIG. 1: DETERMINATION OF ΛMAX OF AZTREONAM IN ALF SOLUTION AT DIFFERENT CONCENTRATIONS

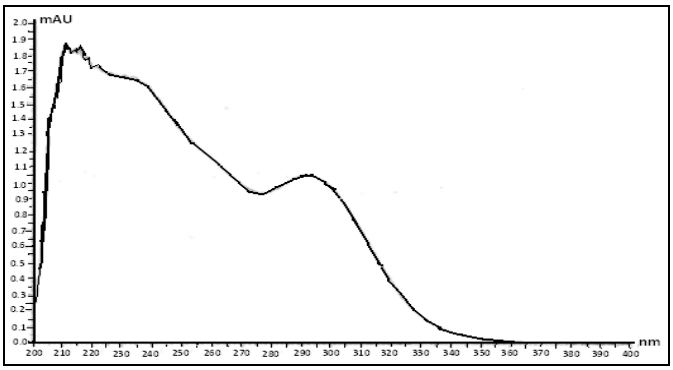

FIG. 2: DETERMINATION OF ΛMAX OF AZTREONAM IN GAMBLE SOLUTION

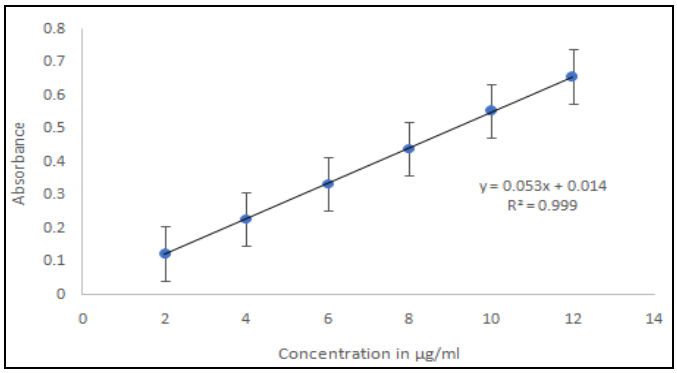

FIG. 3: CALIBRATION CURVE OF AZTREONAM IN ALF SOLUTION

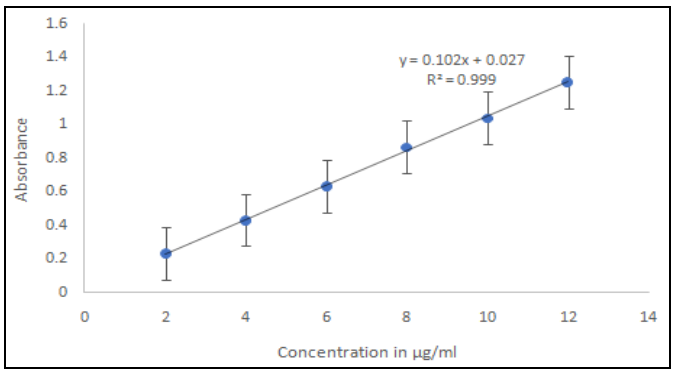

FIG. 4: CALIBRATION CURVE OF AZTREONAM IN GAMBLE SOLUTION

The calibration curves **Fig. 3** and **Fig. 4** showed linearity over a concentration range of 2 to 12 µg/ml. The equations expressed the linearity of the graph A = 0.0536C + 0.0146 and A = 0.1022C + 0.0275 for ALF and Gamble solution respectively, where A and C represents absorbance and

concentration. The correlation coefficients of the curves obtained with linear regression analysis were 0.9995 for ALF solution and 0.9999 for gamble solution.

The data obtained from different dilutions of Aztreonam in ALF and Gamble solution are subjected to regression analysis as shown in **Table 2**. Each dilution was assayed in triplicate, and the correlation coefficients were found to be 0.9995 for ALF and 0.9999 for gamble solution. Hence, the relationship between the concentration and absorbances showed linearity in the range of 2-12µg/ml dilutions of aztreonam in ALF and Gamble solution. The LOD was found to be 0.38 µg/ml and 0.18 µg/ml for ALF and Gamble solution respectively.The LOQ was found to be 1.15 µg/ml and 0.53 µg/ml for ALF and gamble solution respectively as shown in **Table 2**. The significantly low value of LOD and LOQ proved the sensitivity of the process.

TABLE 2: REGRESSION ANALYSIS

Statistical parameters	ALF solution	Gamble solution
Linearity equation	A = 0.0536C + 0.0146	A = 0.1022C + 0.0275
Slope	0.0536	0.1022
Intercept	0.0146	0.0275
Linearity range	2-12µg/ml	2-12µg/ml
Correlation coefficient	0.9999	0.99955
SE of intercept	0.00285	0.011762
SE of slope	0.003061	0.012634
Accuracy	100.15 ± 1.21	99.98 ± 1.47
LOD	0.175466	0.379791
LOQ	0.531716	1.150881

The interday and within day variation study of six different dilutions of Aztreonam solutions in both ALF and Gamble solution showed % RSD less than 2%as shown in **Table 3**. A good accuracy of the repeatability was verified with a mean recovery of more than 99.9% within a day and 99.9 % inter-day variation study of the different dilutions of Aztreonam in ALF and gamble solution.

TABLE 3: INTERDAY AND WITHIN DAY STUDY

Type of solution	Precision		Accuracy	
	% RSD		%	
	Inter-day	Within-day	Inter-day	Within-day
ALF Solution	1.94	1.87	99.80 ± 1.94	99.95 ± 1.87
Gamble solution	1.51	1.9	99.94 ± 1.51	99.97 ± 1.51

The results showed that the recovery study to determine the accuracy of aztreonam in ALF and gamble solution was satisfactory and the % RSD was found to be less than 2% as given in **Table 4**.

TABLE 4: RECOVERY STUDY

Type of solution	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml) n=3	% Recovered	% RSD
ALF	10	8	7.93	99.13	1.18
	10	10	10.06	100.63	0.94
	10	12	11.99	99.96	0.73
Gamble	10	8	8.20	102.55	1.25
	10	10	9.89	98.90	1.16
	10	12	11.99	99.98	0.95

Specificity was determined by analyzing marketed sample of Aztreonam (Azenam 500 mg injection from Aristo Pharmaceuticals Pvt. Ltd., India). The content was found at an average of 98.66 ± 0.37 % in ALF solution and 98.53 ± 0.19 % in gamble solution. The % RSD was found to be 0.378 and 0.198 for ALF and gamble solution respectively. The excipients present in marketed injection did not interfere in the analysis. The results proved specificity of the proposed methods for identification of Aztreonam. Considering a slight change in pH and wavelength % RSD was found to be less than 2% as shown in **Table 5**. This proves the robustness of the method under variable conditions.

TABLE 5: DETERMINATION OF ROBUSTNESS

Parameters	ALF solution		Gamble solution	
	Variation	% RSD	Variation	% RSD
pH	4.7	1.28	7.5	1.59
Wavelength	292	1.58	292	1.94

CONCLUSION: The proposed UV spectroscopic method for analysis of Aztreonam in simulated lungs fluid proved to be sensitive, accurate, precise, specific and robust.

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CONFLICT OF INTEREST: The author declares that there is no conflict of interest.

REFERENCES:

1. https://pubchem.ncbi.nlm.nih.gov/compound/5742832
2. Prakash V, Suresh J and Konari SN: RP-HPLC Forced degradation studies of aztreonam in the pharmaceutical dosage form. Pharm Methods 2018; 9(1): 40-44.
3. Andressa LF and Herida RNS: Validation of a green analytical method for the quantitative analysis of anti-microbial a-Aztreonam in lyophilized powder for injection by Fourier-Transform Infrared Spectroscopy (FT-IR). EC Microbiology 2017; 5: 254-265.
4. Mahgoub H: Spectrophotometric and fluorometric determination of aztreonam in bulk and dosage forms. J Pharm Biomed Anal 2003; 31(4): 767-774.
5. Yang XF, Xu Y, Qu DS and Li HY: The influence of amino acids on aztreonam spray-dried powders for inhalation. Asian J Pharm Sci 2015; 10(6): 541-548.
6. Marques MRC, Loebenberg R and Almukainzi M: Simulated biologic fluids with possible application in dissolution testing. Dissolution Technol 2011; 7: 15-28.
7. Spitler G, Spitz H, Glasser S, Hoffman MK and Bowen J: *In-vitro* dissolution of uranium-contaminated soil in simulated lung fluid containing a pulmonary surfactant. Health Phys 2015; 108(3): 336-43.
8. ICH Topic Q2 (R1) Validation of Analytical Procedures: Text and Methodology. Int Conf Harmon 2005.
9. Rajput SJ and Sathe MA: Application of doe and statistical analysis for development and validation of analytical method for chlorhexidine gluconate and cetrimide in its bulk and pharmaceutical dosage forms. Int J Pharm Sci Res 2018; 9(7): 2800-2806.
10. Ferraz RS, Mendonca EAM, Silva JPA, Cavalcanti IMF, Nougurira M, Galdino AL, Pitta IR, Lima M and Magalhaes: Validation of a UV spectrophotometric analytical method for determination of LPSF / AC04 from inclusion complex and liposomes. Braz J Pharm Sci 2015; 51(1): 183-191.
11. Sing P, Chaudhari VK, Verma PK, Singh AK and Yadav VK: Development and validation of the UV-visible spectro-photometric method for the determination of levofloxacin in bulk and tablet formulation. Int J Res Dev Pharm L Sc 2015; 4(1): 1375-1378.
12. Das J and Dhua M: UV spectrophotometric assay method development and validation of metronidazole in bulk and tablet formulation. J Pharmsci Tech 2014; 3(2): 106-109.
13. Dange YD, Honmane SM, Bhinge SD, Salunkhe VR and Jadge DR: Development and validation of UV spectro-photometric method for estimation of metformin in bulk and tablet dosage form. IJPER 2017; 51(4S): S754-S760.
14. Salgado HRN and Cazedey ECL: Development and validation of UV spectrophotometric method for Orbifloxacin assay and dissolution studies. Braz J Pharm Sci 2014; 50(3): 457-65.

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