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Spectrophotometric and fluorimetric determination of aztreonam in bulk and dosage forms

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Abstract

Spectrophotometric and fluorimetric determination of aztreonam were achieved through its reaction with cerium (IV) in acidic medium. The spectrophotometric method involves the quantitation of the amount of ceric equivalent to aztreonam by measuring the absorbance at 317 nm and the corresponding first-derivative value at 284 nm for the blank solution against the reaction solution. Beer's law is obeyed over the concentration ranges of 1.5–4 and 1–4 μg ml^{-1}, respectively. Meanwhile, in the fluorimetric method, higher sensitivity was achieved by measuring the fluorescence intensity of the formed cerium (III) at $\lambda_{em} = 357$ nm ($\lambda_{ex} = 257$ nm) within a concentration range 150–350 ng ml^{-1}. Study of the reaction conditions and reaction stoichiometry were presented. Interference from L-arginine, which is frequently co-formulated with aztreonam, was tested. The proposed procedures were applied successfully to the determination of aztreonam in pure form and in presence of arginine both in laboratory mixtures and commercial vials. The proposed methods are sensitive, accurate and precise as compared with the official USP 24 HPLC method.

Keywords: Aztreonam; Spectrophotometry; Fluorimetry; Pharmaceutical analysis; Cerium

1. Introduction

Aztreonam is a synthetic monocyclic β-lactam antibiotic highly active against a broad range of gram-negative organisms [1]. Aztreonam is the subject of a monograph in the USP 24 [2] whereby HPLC method is recommended for its determination in raw material and in parentral formulations. A number of analytical techniques have been utilized for the quantitation of aztreonam in dosage forms and/or in biological fluids. Among these are HPLC [3–6], mass spectrometry [7], electroanalytical [8,9] and microbiological [10] techniques.

Aztreonam has also been determined using spectrophotometric methods of analysis. Some application procedures are based on absorbance measurements in the UV region either directly [11] or after complexation of aztreonam with Cu (II) [12]. Operating in the mode of derivatives has also been utilized [11–13]. Measurements in the visible region have been applied after derivatization reaction with reagents such as sodium nitrite [14] or hydroxylamine [15]. Spectrophotometry with flow injection operation has also been published

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[16]. The chemiluminescence of aztreonam and other β-lactam antibiotics based on the luminol reaction has been investigated by a FIA technique [17].

In the analytical literature reviewed for aztreonam no, up to date, references were described utilizing spectrofluorimetric technique for the quantitation of aztreonam. Thus, one of the important aims of the present work is to establish a sensitive fluorimetric procedure for the evaluation of this antibiotic.

Cerium (IV) is a versatile oxidimetric reagent. It has been used in manual or in flow-injection analysis of pharmaceutical compounds such as paracetamol [18], promethazine [19], penicillamine [20], ritodrine HCl [21] and cephalosporins [22].

The present paper describes two methods, spectrophotometric and fluorimetric, for the determination of aztreonam after its reaction with ceric ions in acid medium. Both methods are satisfactorily applied for aztreonam determination in pure form and in pharmaceutical injections with no interference from co-formulated substances.

2. Experimental

2.1. Apparatus

Absorbance was measured on a Perkin-Elmer-Lambda EZ 201 spectrophotometer equipped with a Panasonic 24 pin KX-P 3626 printer. The fluorimetric measurements were performed on a Perkin-Elmer 650-10S fluorescence spectrometer. The latter apparatus was a gift from the Alexander Von Homboldt Foundation (Bonn, Germany) to Prof. A.M. Wahbi.

2.2. Materials and reagents

Aztreonam (SQ-026776) authentic powder was provided by Bristol-Myers Squibb, Pharmaceutical Research Institute, NJ, USA. The drug substance was used as a reference standard.

Azactam, sterile powder for injection (a blend of aztreonam and L-arginine, containing approximately 780 mg L-arginin per gram aztreonam), was obtained from commercial sources.

L-arginine monohydrochloride, Fluka Chemie AG, Switzerland.

Cerium (IV) solutions were prepared by dissolving the appropriate quantities of ceric sulphate (BDH, Poole, England), in 2 M sulphuric acid (BDH, Poole, England), so as to obtain 1×10^{-3} M (solution A) and 1×10^{-4} M (solution B) solutions.

2.3. Preparation of standard solutions

An accurately weighed quantity of aztreonam (25 mg) was dissolved in distilled water in a 100 ml calibrated flask. Aliquots of the above prepared stock solution were further diluted to obtain 25 μg ml^{-1} (standard solution A) and 2.5 μg ml^{-1} (standard solution B) working standard solutions.

2.4. Preparation of sample solutions

The contents of one Azactam vial for injection were quantitatively transferred to 500-ml volumetric flask. The powder was dissolved and the volume was completed with distilled water. Suitable dilutions were made to obtain the applicable concentration range for the spectrophotometric and fluorimetric procedures.

2.5. General procedure

An aliquot of aztreonam aqueous solution was transferred into a 10-ml volumetric flask, to which 2 ml cerium (IV) solution was added. The flask was heated in a thermostated water-bath at 100 °C for 25 min, cooled and diluted to the mark with distilled water.

2.5.1. Spectrophotometric measurement

The absorbance at 317 nm and the first derivative, D_1-value, at 284 nm were measured for the blank solution against the experimental reaction solution.

2.5.2. Fluorimetric measurement

The relative fluorescence intensity was measured for the reaction solution at emission wavelength of 357 nm with the excitation wavelength at 257 nm.

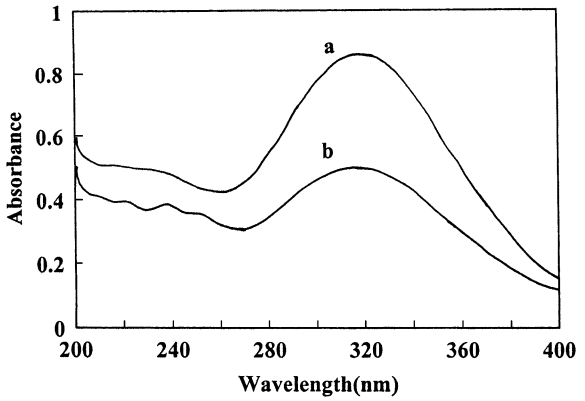


Fig. 1. Absorption spectra of 2×10^{-4} M cerium (IV) sulphate reagent solution in sulphuric acid before (a) and after (b) reaction with aztreonam.

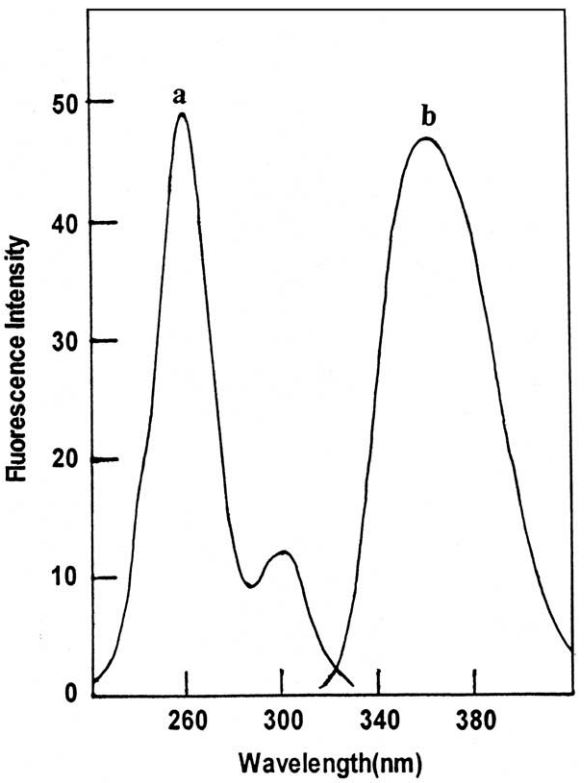


Fig. 2. (a) Excitation and (b) emission spectra of cerium (III) generated after reaction with aztreonam.

2.6. Preparation of calibration graphs

The general procedure was followed, using aliquots of 0.4–1.6 ml from standard solution A and 2 ml of Ce (IV) solution A for the spectrophotometric procedure. Whereas for the fluorimetric procedure, 0.6–1.4 ml aliquots from standard solution B and 2 ml of Ce (IV) solution B were used.

3. Results and discussion

Cerium (IV) is a strong oxidizing agent that has been used in the determination of several drugs. Its acidic solution is yellow in color and has a maximum absorbance at 317 nm. Its reduced form, cerium (III), is colorless and possesses native fluorescence at $\lambda_{em} = 357$ nm ($\lambda_{ex} = 257$ nm).

Oxidation reaction of aztreonam with Ce (IV) ions is the basis of two analytical procedures (spectrophotometric and fluorimetric) developed for aztreonam determination. The reaction is monitored either by the decrease in the absorbance value of cerium (IV), at 317 nm (Fig. 1) or by the fluorescence intensity, of cerium (III) ions, at $\lambda_{em} = 357$ nm and $\lambda_{ex} = 257$ nm (Fig. 2) developed after the addition of the drug.

After optimizing the reaction conditions, quantitative determination of the drug can be performed. The drug solution is treated with a known excess of ceric sulphate reagent in acidic medium with subsequent measurement of three signals. The spectrophotometric signals are the absorbance value (A) at 317 nm and the corresponding first-derivative value (D_1) at 284 nm measured for the reagent solution against the reaction solution. This gives the amount of ceric (IV) ions that has reacted with aztreonam. The fluorimetric signal is the fluorescence intensity (F) of the reaction solution measured at $\lambda_{em} = 357$ nm ($\lambda_{ex} = 257$ nm). This gives the cerous (III) ions generated equivalent to the drug.

3.1. Optimization of reaction conditions

A series of experiments was conducted to establish the optimum experimental variables at

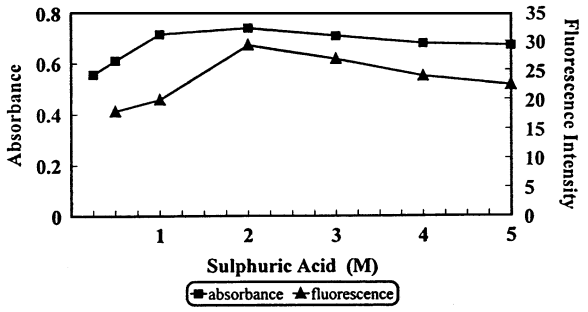


Fig. 3. Effect of sulphuric acid concentration (M) on absorbance and fluorescence intensity measurements.

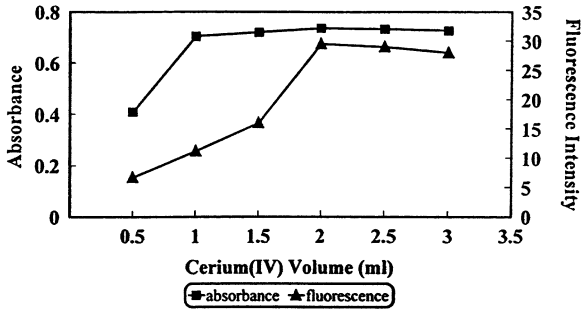


Fig. 4. Effect of cerium (IV) concentration, volume of 1×10^{-3} and 1×10^{-4} M solutions in 2 M sulphuric acid, on absorbance and fluorescence intensity measurements, respectively.

which maximum and reproducible analytical signals were achieved. The parameters optimized included the effect of acidity, ceric ions, temperature and time.

3.1.1. Effect of sulphuric acid

The oxidation of aztreonam with Ce (IV) was studied in acid medium. Sulphuric acid was selected for the solubility and stability of ceric sulphate. The effect of sulphuric acid concentration on the reaction was examined in the range 0.5–5 M H_2SO_4 (Fig. 3). The analytical signal increases with increasing H_2SO_4 concentration reaching its optimum value at 2.0 M concentration after that it starts to decrease.

3.1.2. Effect of Ce (IV) ion concentration

The effect of Ce (IV) ions concentration (volume of 1×10^{-3} and 1×10^{-4} M ceric sulphate solution in 2 M H_2SO_4) on the resulting spectrophotometric and spectrofluorimetric signals, respectively, is shown in Fig. 4. Maximum signals were obtained with 2.0-ml volume of the respective concentration of the reagent. With 4.0-ml volume and rising, higher unacceptable absorbance values were obtained. In the spectrofluorimetric measurement, the high background of the blank was the limiting factor since Ce (III) ions were always present to some extent in the Ce (IV) solution.

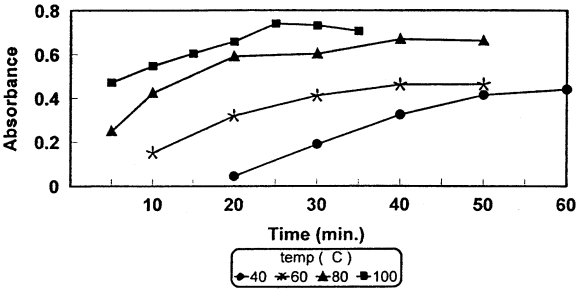


Fig. 5. Effect of temperature and heating time on absorbance measurements (aztreonam = 4 μg ml^{-1} except for temperature 40 °C = 10 μg ml^{-1}).

3.1.3. Effect of temperature and heating time

The oxidation reaction of aztreonam with Ce (IV) was carried out at different temperatures (ambient temperature, 40, 60, 80 °C and boiling water bath) using a thermostated water bath (Fig. 5) for periods ranging from 5 to 60 min. Preliminary experimental results revealed that the reaction is strongly dependent on temperature and time.

At ambient temperature ($\sim$23 °C) the reaction rate proceeds slowly. Detectable signals are shown after a period not less than 60 min and using high concentration of the drug ($\geqslant$1 mg (100 ml)$^{-1}$). However, heating the reaction solution was found to increase both reaction rate and signal sensitivity. The recommended condition was heating in a boiling water bath for 25 min (Fig. 5).

The final reaction solutions were allowed to stand under normal laboratory conditions, the maximum signals (A, D_1 and F) obtained were found stable for a least 30 min.

Table 1
Statistical data of the calibration graphs for the determination of aztreonam using the proposed methods

Method	Linearity range	Wavelength (nm)	Regression equation[a]		Standard deviation		Correlation coefficient (r)	Variance (S_b^2)	Limit of detection (μg ml^{-1})
			Intercept (a)	Slope (b)	Intercept (S_a)	Slope (S_b)			
Spectrophotometric									
Absorbance	1.5–4.0 μg ml^{-1}	317	5.590×10^{-3}	18.554×10^{-2}	0.647×10^{-2}	0.225×10^{-2}	0.9997	0.470×10^{-2}	7.231×10^{-2}
First derivative	1.0–4.0 μg ml^{-1}	284	-1.000×10^{-4}	14.960×10^{-2}	0.552×10^{-2}	0.194×10^{-2}	0.9997	0.469×10^{-2}	1.794×10^{-4}
Spectrofluorimeric	150–350 ng ml^{-1}	$\lambda_{em} = 357$ $\lambda_{ex} = 257$	-19.701×10^{-2}	10.107×10^{-2}	55.850×10^{-2}	0.206×10^{-2}	0.9992	35.176×10^{-2}	9.466×10^{-3}

[a] Number of standard samples, $n = 6$.

3.2. Method validation

3.2.1. Linearity of the methods and detection limits

Under the optimized reaction conditions, the spectrophotometric (A and D_1) and the fluorimetric (F) signals measured at the specified wavelengths (Table 1) were found to be proportional to the concentration of aztreonam in the concentration ranges given in Table 1. Using the method of least squares, regression analysis was made for the intercepts, slopes, correlation coefficients and variances (Table 1). Separate determinations at different concentration levels were carried out for standard aztreonam to test reproducibility of the (A), (D_1) and (F) values. The relative standard deviations (RSD) were found to be less than 2%.

The detection limits varies from 1.794×10^{-4} to 7.231×10^{-2} μg ml^{-1} as shown in Table 1.

3.2.2. Accuracy and precision

In order to determine the accuracy and precision of both procedures, solutions containing two different concentrations of aztreonam were prepared and analyzed in five replicates. The analytical results obtained from the investigation are summarized in Table 2. The percentage relative error (Er%) and the percentage relative standard deviation (RSD%) are considered very satisfactory.

3.2.3. Interference studies

Aztreonam for injection is a dry mixture of sterile aztreonam and L-arginine [2]. The influence of L-arginine was investigated, side by side, under the same conditions proposed for aztreonam. No reaction was observed between L-arginine and Ce (IV). In addition, different standard mixtures containing aztreonam and arginine in concentration ratio 1:1 were assayed for azreonam by the spectrophotometric and spectrofluorimetric procedures. No significant interferences could be observed from arginine since good and accurate results were obtained for aztreonam.

Table 2
Accuracy and precision for the determination of aztreonam using the proposed methods

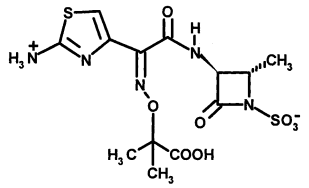
Method	Nominal value	Found ± S.D.[a]	Er %[b]	RSD %[c]
Spectrophotometric				
Absorbance	2 μg ml^{-1}	2.04 ± 0.020	2.0	0.98
	4 μg ml^{-1}	3.98 ± 0.026	0.5	0.65
First derivative	2 μg ml^{-1}	2.03 ± 0.010	1.5	0.49
	4 μg ml^{-1}	4.04 ± 0.041	1.0	1.01
Spectrofluorimetric	200 ng ml^{-1}	201.00 ± 0.402	0.50	0.20
	300 ng ml^{-1}	300.93 ± 0.842	0.31	0.28

[a] Mean ± S.D. for five determinations.
[b] Percentage relative error.
[c] Percentage relative S.D.

3.2.4. Analysis of commercial pharmaceutical preparation

The proposed procedures were further applied to the analysis of aztreonam in azactam vials containing L-arginine. The results in Table 3 are in accordance with those obtained by the official HPLC procedure [2]. Statistical analysis of the results by using the Student's *t*-test and variance ratio *F*-test (Table 3) showed no significant difference between the performance of the compared methods as regards to accuracy and precision. For more confirmation the standard addition method was applied to test the reliability of the proposed methods by adding known quantities of pure drug to the previously analyzed vials. The results (% recoveries ± S.D.) summarized in Table 3 confirm that good accuracy and precision can be obtained.

3.3. Reaction stoichiometry and proposal of reaction pathway



Aztreonam

It has been reported that aztreonam is susceptible to hydrolysis with subsequent opening of the

Table 3
Assay results for aztreonam in vials using the proposed methods

	Spectrophotometric		Spectrofluorimetric	USP 24 (HPLC)
	Absorbance	First derivative		
Azactam vials				
Recovery (%)[a]	99.71	100.40	99.81	100.06
± S.D.	0.31	0.22	0.88	0.41
t (2.31)[b]	1.52	1.63	0.58	
F (6.39)[b]	1.75	3.47	4.61	
Standard addition				
Recovery (%)[a]	100.02	100.00	99.90	
± S.D.	0.27	0.90	1.03	

[a] Refers to the average of five measurements.
[b] Values in parenthesis are the theoretical values at $P = 0.05$.

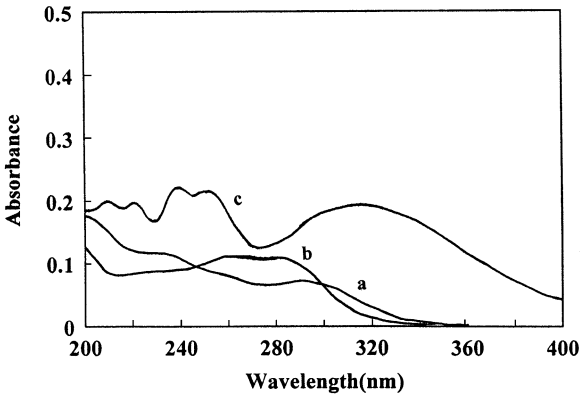


Fig. 6. UV absorption spectra for (a) aztreonam in water; (b) aztreonam after heating with 2 M H_2SO_4 and (c) aztreonam after oxidation reaction with Ce (IV) in presence of 2 M H_2SO_4.

β-lactam ring in acidic aqueous solution [23,24]. This fact supports that the reaction of aztreonam with Ce (IV) in acidic solution; 2 M H_2SO_4, most propably follows oxidative degradation pathway. In this concern, the UV spectra for aztreonam in water (Fig. 6a), aztreonam in 0.4 M H_2SO_4, after heating with 2 M H_2SO_4, (Fig. 6b) and aztreonam after oxidation reaction with Ce (IV) in presence of 2 M H_2SO_4 (Fig. 6c) were compared. As can be seen that aztreonam is not a strong absorbing compound which exhibits two minor peaks at about 288 and 237 nm (Fig. 6a). The acid degradation product (Fig. 6b) nearly keeps the same spectral features with minor changes which can be attributed to β-lactam ring opening. Possible decarboxylation step could be suggested in the view of the degradation of structurally related compounds, penicillins [25]. An apparent wavelength shift to the shorter side was observed in the UV spectrum of the oxidation reaction product (Fig. 6c). This matter agrees with the proposed oxidation of the sulphur atom of the thiazole ring to sulfone.

The stoichiometry of the reaction under the experimental reaction conditions was studied; the molar ratio was found to be 1:4 [aztreonam:Ce (IV)]. Such a finding is in agreement with the referred oxidation product.

4. Conclusion

Although aztreonam has been determined by a variety of techniques, the proposed procedures described here are rapid, simple and can be used in the control laboratory.

A comparison of the proposed methods shows all of them are equally accurate and precise. The spectrophotometric measurement in the derivative mode is a little more sensitive than the zero-order absorbance measurement. Moreover, the fluorimetric procedure offers the most sensitive measurement that could be adopted for the assay of aztreonam in biological samples.

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