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Utilization of colorimetric and atomic absorption spectrometric determination of aztreonam through ion pair complex formation

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Abstract

Three rapid and sensitive, colorimetric and atomic absorption spectrometric methods were developed for the determination of aztreonam. The proposed methods depend upon the reaction of cobaltthiocyanate (I) or reineckate (II) ions with the drug to form stable ion-pair complexes which extractable with chloroform. The greenish blue and pink color complexes are determined either colorimetrically at λ_{max} 625 and 525 nm for I and II reagents, respectively, or by atomic absorption spectrometry, directly using the organic extracted complex, or indirectly, using the supernatant. The three procedures are applied for the determination of aztreonam in pure and in pharmaceutical dosage forms applying the standard additions technique and the results obtained agreed well with those obtained by the official method.

Keywords: Aztreonam determination; Ion-pair complex; Colorimetry; Atomic absorption spectrometry

1. Introduction

Aztreonam [78110-38-0] is a monocyclic β-lactam antibiotic characterized by its excellent efficacy against gram-negative micro-organisms.

Most published methods for the determination of aztreonam, both in pharmaceutical products and in biological fluids, use high-performance liquid chromatography (Meulemans et al., 1986; Ehret et al., 1987; Wahbi et al., 1988; Pilkiewicz et al., 1993). Spectrophotometric methods have been used to a certain extent for the determination of penicillins (Amin et al., 1994; Yang et al., 1999) and cephalosporins (Issa et al., 1996; Mishra et al., 1998; Xu et al., 1998; Ayad et al., 1999). For aztreonam, direct ultraviolet measurement has been performed using the derivative mode (Mohamed et al., 1988a; Morelli et al., 1990). Derivatization procedures have also been used with reagent such as hydroxylamine (Mohamed et al., 1988b) and sodium nitrite (Uri and Jain, 1985, 1986), which permit measurement of the visible region. Batch and flow injection spectrophotometric method based on the reaction with hydroxylamine to form hydroxamic acid and subsequent

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reaction with iron (III), giving a red complex absorb maximally at 496 nm (Martin et al., 1992). The last work on aztreonam was the polarographic determination (Pewrez et al., 1993) using differential puls technique. Yet, no work has performed to its determination.

The purpose of the present investigation is to develop a simple spectrophotometric and atomic absorption spectrometric methods for the determination of aztreonam and to apply the procedures to various dosage forms. The methods are based on ion-pair complex formations between the drug and ammonium cobaltthiocyanate (I) or reineckate (II).

2. Materials and methods

2.1. Apparatus

The pH values of solutions were measured using an Orion Research Model 601A/Digital Ionalyzer pH-meter. The absorption spectra for all measurements was carried out using a Perkin-Elmer λ3B spectrophotometer. The atomic absorption measurements for the determination of metal ion were carried out using a Hitachi atomic absorption Z-6100 polarized Zeeman spectrometer. For AAS, the cobalt and chromium were measured at wavelengths 240.73 and 357.87 nm, respectively, slit width, 0.2 nm, relative noise, 1.0, detection limit, 0.01 μg ml^{-1}, linear dynamic range, 0.01–100 μg ml^{-1}, lamp current, 5 mA and integration time, 3 s. The flame used was the acetylene-air mixture.

2.2. Reagents

Analytical grade reagents and double distilled water was used to prepare all solutions. Aztreonam was obtained from ICN Biomedical. A stock solution (1×10^{-3} M) was prepared by dissolving 43.3 mg of the solid in 100 ml of double distilled water. The pharmaceutical dosage forms were obtained from the local market.

Ammonium cobaltthiocyanate reagent (I) [5×10^{-3} M] was prepared by dissolving 11.9 g of cobalt chloride hexahydrate and 28.13 g of ammonium thiocyanate in 100 ml of water (Nerin et al., 1985). 5×10^{-3} M Ammonium reineckate (II) (Aldrich product) solution was also prepared by dissolving appropriate weight in 100 ml bidistilled water.

Acetate buffer solutions of pH values 2.11–5.66 were prepared by mixing appropriate volume of 1.0 M acetic acid with 1.0 M sodium acetate as recommended (Britton et al., 1952).

2.3. General procedures

2.3.1. Spectrophotometric procedure

An aliquot containing 25–700 μg of aztreonam was transferred into a 50 ml separating funnel, 5.0 ml of 5×10^{-3} M reagents I or II and 10.0 ml buffer solution of the optimum pH value (Table 1) were added successively. The volume of aqueous phase was adjusted to 20 ml with bidistilled water. Equilibrate the solution with 20 ml of chloroform by shaking for 2.0 min. The chloroformic layer was separated and clarified by centrifugation at 8000 rev min^{-1} for 5.0 min. Into a 25 ml calibrated flask, the upper layer solution was filterate through a dry filter-paper moistened with choroform, washed, if necessary, with chloroform and adjust to the mark with the same solvent. The absorbance of solutions were measured at 625 and 525 nm using reagents I and II, respectively, against a blank solution prepared using 5.0 ml of water instead of the drug solution. The calibration graph was obtained by applying the same procedure, using standard drug solutions.

2.3.2. Atomic-absorption spectroscopic procedures

2.3.2.1. Direct method. Proceed as above 'and adjust to the mark with the same solvent'. The chloroformic layer is evaporated to dryness on boiling water bath and the residue was dissolved in isobutylmethylketone, and completed to the mark with the same solvent. This solution is then aspirated directly in the atomic absorption spectrometer and measure the metal ions concentration in the organic solvent. Calculate the concentration of the tested drug from the relevant calibration graph.

2.3.2.2. Indirect method. Proceed as above 'the chloroformic layer was separated'. The aqueous layer is then diluted and aspirated directly in the atomic absorption spectrometer. The metal ion consumed in the formation of ion pairs was calculated. Each 0.1 ml 5×10^{-3} M of cobaltthiocyanate is equivalent 17.4 μg of aztreonam, whereas each 0.1 ml of 5×10^{-3} M of reineckate is equivalent to 8.71 μg of the same drug.

2.3.3. For pharmaceutical dosage forms (ampoules)

Ten ampoules were weighted and calculate average weigh of each one. A quantity equivalent to 25 mg of the drug was dissolved in water, and completed to 250 ml in calibrated flask with water. A total of 0.08 μl of the prepared solution was used for the drug determination by the standard additions method utilizing the three prior procedures.

3. Results and discussion

According to Babko et al. (1968) a large number of analytically important complexes consist of the system metal ion-electronegative ligand-organic base. Most of these complexes are extractable in the usual organic solvents such as hydrocarbons and the halogentated derivatives.

Aztreonam is found to react with cobaltthiocyanate (I) and reineckate (II) ions to form stable ion pair complexes. These complexes are sparingly soluble in aqueous solution, but are readily extractable in organic solvents.

3.1. Optimization of reaction variables

Investigations were carried out to establish the most favorable conditions for the ion pair complex formation of aztreonam with cobaltthiocyanate (I) or reineckate (II) ions to achieve maximum color development in the determination of the drug. The influence of some variables on the reaction has been tested as follow:

3.1.1. Effect of reagent concentration

Experiments was carried out in which the volume of the aqueous layer was kept constant at 20 ml while the concentration of reagent was increased revealed that 5.0 ml of the reagent is the optimum volume (Fig. 1). The excess reagent used is probably as a result of dissociation in aqueous medium as fraction of the ion-pair formed.

Table 1
Analytical characteristics of the three procedures, precision and accuracy

Parameters	Cobaltthiocyanate			Reineckate		
	Spect.	Direct AAS	Indirect AAS	Spect.	Direct AAS	Indirect AAS
pH	3.13	3.13	3.13	3.47	3.47	3.47
λ_{max}	625	240.73	240.73	525	357.87	357.87
Range of determination (μg ml^{-1})	1.0–28.0	17.4–121.8	17.4–139.2	1.0–22.0	8.7–60.9	8.7–87.0
Detection limits (μg ml^{-1})	0.02	0.087	0.087	0.015	0.044	0.044
Range of errors (%)	±1.6	±0.9	±1.1	±1.8	±1.2	±1.4
Regression equation[a]						
Intercept	+0.009	+0.007	−0.010	−0.013	+0.015	+0.007
Slope	0.027	0.013	0.013	0.024	0.011	0.011
Correlation coefficient (r)	0.9996	0.9999	0.9999	0.9992	0.9998	0.9998
Calculated t-value (2.57)[b]	1.27	0.93	1.09	1.43	1.16	1.31
Calculated F-value (5.05)[b]	1.81	1.57	1.72	2.18	1.75	2.08

[a] $A = a + bC$, where C is the concentration in μg ml^{-1}.
[b] Theoretical values for five degrees of freedom and 95% confidence limits.

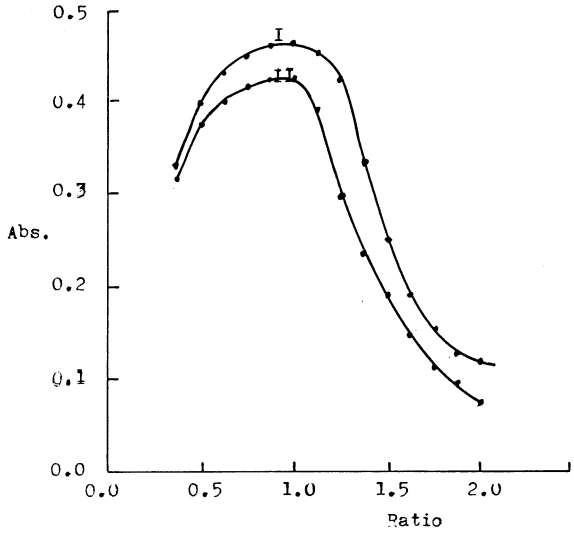

Fig. 1. Effect of reagent concentration on complexation of reagent I and II with 18 μg/ml^{-1} of azotreonam.

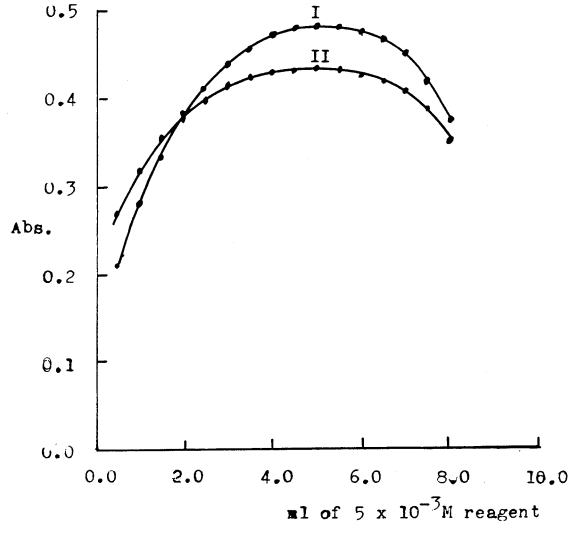

Fig. 2. Effect of phase volume ratio on the extraction of 18 μg ml^{-1} aztreonam using reagents I and II.

3.1.2. Effect of pH

The pH variation of the aqueous phase between 2.11 and 5.66 showed that the extraction of the complex is a maximum at pH range 2.85–3.45 and 3.25–3.78 using cobaltthiocyanate and reineckate ions, respectively. The pH 3.13 and 3.47 were selected for further study on using the cobaltthiocyanate and reineckate, respectively, since the results are highly concordant at those pH values. The amount of buffer added to the total volume of 20 ml aqueous layer was found to be 10 ml that gave marginally the best results.

3.1.3. Effect of solvent

The effect of extracted solvent showed that, the polarity of the solvent affects both extraction efficiency and absorbance intensity. The results using different extraction solvents applying reagents I and II indicated that the response using chloroform and benzene are useful solvent for both reagents. The former was selected because of its slightly higher sensitivity and considerably lower extraction ability for the reagents. Reproducible absorbance readings were obtained after a single extraction with 20 ml of chloroform. The over-all extraction efficiency was 99.7%. Repeated extraction did not show any increase in the recovery percent results. Also, washing of the chloroformic extract with water was not practicable as the feebly stable ion-pair complex would decompose to the equated form, the color was discharged.

3.1.4. Effect of phase volume ratio

Experiments were performed in which the volume of chloroform was kept constant at 20 ml while that of the aqueous phase varied between 15–50 ml, keeping the reagent and aztreonam content constant as in evident from Fig. 2. The absorbance increase on increasing the chloroform ratio until achieved maximum absorbance at 1:1 ratio, then, the absorbance decrease gradually with increasing the aqueous phase to chloroform phase volume after achieving (1:1) ratio. However, ratio 1:1 (aqueous : chloroform) was chosen so as to compromise between maximum absorbance and colorless blank; thus accordingly a linear relationship between absorbance and concentration can be achieved.

3.2. Stoichiometry of the reaction

In order to study the stoichiometry of the reaction, the molar ratio between reagents I or II and aztreonam was determined using the continuous

variations method. As is evident from Fig. 3, the ratio between cobaltthiocyanate and aztreonam is 1:2, whereas for reineckate is 1:1 (reagent:drug). The shape of the curves indicated that the complexes were labil, hence a large excess of reagents I or II must always be used to enhance the formation of the complex. Accordingly, the ion pair complex formation reaction can be proposed as follow.

$$[Co(SCN)_4]^{2-} + [Drug] \rightarrow [Co(SCN)_4][Drug]_2$$

$$[Cr(NH_3)_2(SCN)_4]^{-} + [Drug]$$

$$\rightarrow [Cr(NH_3)_2(SCN)_4][Drug]$$

The developed colors are very stable, their intensities remain constant even after several days.

3.3. Optimization for AAS measurements

It was not practical to aspirate the chloroformic solution of the ion-pair complex in the atomic absorption spectrometer. It is better to extract the ion-pair complex with chloroform, evaporate, and then dissolve the residue in isobutylmethylketone which can be aspirated directly to the atomic absorption spectrometer. On the other hands, the metal ions [Co^{2+} or Cr^{3+}] consumed in the formation of ion pair was calculated indirectly by measuring the metal ion content in the aqueous phase directly without extraction of the formed ion-pair or farther treatment using atomic absorption spectrometer.

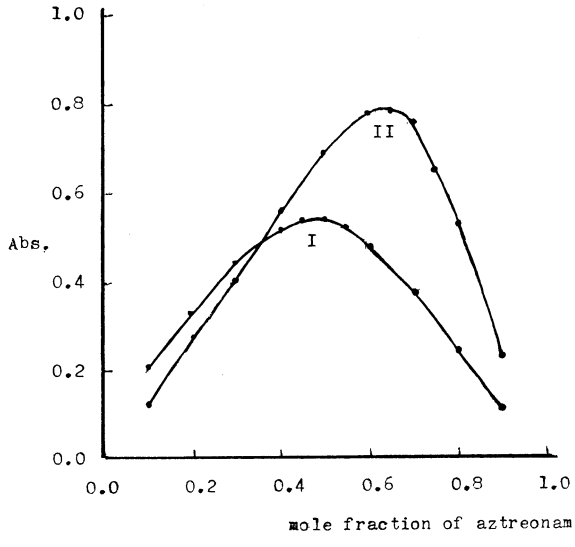

Fig. 3. Continuous variation plot for aztreonam ion pair formed with reagents I and II.

3.4. Qunatification

Beer's law is valid within the concentration range 1.0–28 and 1.0–22 μg ml^{-1} of aztreonam using reagents I and II, respectively. For more accurate analysis, Ringbom optimum concentration range was calculated to be 2.0–26 and 2.0–20 μg ml^{-1}, respectively, for I and II reagents. The molar absorptivity and Sandell sensitivity were 1.16×10^4 l mol^{-1} cm^{-1} and 0.038 μg cm^{-2} using reagent I and 1.04×10^4 l mol^{-1} cm^{-1} and 0.042 μg cm^{-2} using reagent II. The linear regression equations were $A = 0.009 + 0.027C$ ($r = 0.9996$) and $A = -0.013 + 0.024C$ ($r = 0.9992$) for ion-pair of I and II reagents, respectively. The results obtained were compared with those of the official method (United State Pharmacopoeia, 2000) (based on chromatographic technique). Detection limits (3σ) and statistical analysis of the obtained results revealed that there is no significant difference between both as shown in Table 1.

For direct and indirect AAS methods a calibration graphs with good linearity were obtained as recorded in Table 1. The linear regression equations for both methods were also calculated. Correlation coefficient, intercept and slope values for the calibration data of each method were calculated using the least-squares method. Detection limits for both methods were also evaluated and recorded in Table 1.

The performance of the proposed methods was assessed by comparison with the official method (United State Pharmacopoeia, 2000) (based on chromatographic technique). Mean values were obtained with a Student's *t*- and *F*-tests at 95% confidence limits for five degrees of freedom (Miller et al., 1993). The results showed comparable accuracy (*t*-test) and precision (*F*-test), since the calculated values of *t*- and *F*-tests were less than the theoretical data (Table 1).

Table 2
Analytical determination of aztreonam in pure and in dosage forms

Sample	Taken (μg ml^{-1})	Added (μg ml^{-1})	Found[a]						
			Cobalt thiocyanate			Reineckate			Official
			Spect.	Direct AAS	Indirect AAS	Spect.	Direct AAS	Indirect	
Aztreonam	10	–	9.95	–	–	10.10	9.90	10.05	9.85
		5	15.10	–	–	14.85	14.95	15.15	15.25
		10	20.15	19.85	19.90	19.95	20.20	19.80	19.70
		15	24.80	25.15	25.25	–	24.80	24.75	25.35
		30	–	40.25	39.80	–	39.70	40.30	40.60
		60	–	69.50	70.60	–	–	69.40	71.00
		120	–	–	119.10	–	–	–	117.00
Azactam (500 mg ampoule)[b]	8	–	8.05	–	–	7.90	–	–	7.85
		8	15.90	–	–	16.15	16.10	15.85	15.75
		16	24.20	23.75	23.80	–	24.25	23.90	24.50
		32	–	40.30	39.75	–	39.70	40.25	40.70
		64	–	71.00	72.80	–	–	73.10	70.60
		128	–	–	134.50	–	–	–	132.20

[a] Average of six determinations.
[b] Novartis Pharmaceutical Company, Cairo, Egypt.

The reproducibility of the proposed procedures was assessed by running six replicate samples, each containing 15 μg ml^{-1} for spectrophotometric procedure and 37.4 μg ml^{-1} for direct and indirect AAS procedures, of the studied drug in the final assay solution. The relative standard deviations were 0.56, 0.98 and 0.81 on using reagent I, whereas using reagent II were 0.77, 1.13 and 1.02 for spectrophotometric, direct and indirect AAS procedures, respectively.

3.5. Analytical applications

The proposed methods were applied to the determination of aztreonam in pure and dosage forms (ampoule) using the standard additions method. In the dosage forms, the aztreonam is present in combination with L-arginine. The results obtained are given in Table 2 for the analysis of commercial preparations. The proposed methods has the advantage of being virtually free from interference (either from excipients such as lactose, fructose and starch or from common degradation products and L-arginine). Therefore, the standard addition principle was used to evaluate the accuracy of the proposed methods and to test interferences (Table 2).

The proposed methods are simple, rapid, accurate, sensitive and can be easily used for the routine determination of aztreonam.

4. Conclusions

The data obtained above indicate that the proposed methods described for the indirect AAS have advantages of simplicity, time consuming, without extraction into organic solvent (as colorimetric and direct AAS methods) or further treatment (as in direct AAS methods) in addition to wider range of aztreonam determination with higher sensitivity. The reagents utilized in the described methods above can safely be used under the proper conditions for satisfactory analysis of aztreonam in pure form. Regarding to the more sensitive, accurate and precise results, cobaltthiocyanate methods is more better than reineckate method from the point of view wide determination, range, low range of error's, high correlation coefficient (r) values applying colorimetric, direct and indirect AAS methods (Table 1). The proposed methods are suitable for the determination of aztreonam in dosage forms without interferences from excipients such as starch and glucose or from common degradation products, suggesting applications in bulk drug analysis.

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