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Development and Validation of a Ultraviolet (UV) Spectrophotometric Method for Determination of aztreonam in Pharmaceutical Products

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Abstract

Aztreonam, the first agent from the monobactam family to be therapeutically approved, is a monocyclic synthetic antimicrobial with bactericidal activity against Gram-negative bacteria. It is used to treat urinary tract infections, respiratory infections, skin infections and intra-abdominal infections. This paper describes the developed and validated an analytical method to quantify aztreonam cheaply and easily using spectrophotometry in the UV region. The absorption was checked at 292 nm using purified water as solvent. The validation parameters were linearity, selectivity, precision, accuracy, robustness, limit of detection and limit of quantification. The range of linearity and selectivity was 27 - 72 µg/mL, with r smaller than 0.9999. The repeatability of the method was 0.20% and intermediate precision indicated that the difference between the means was statistically insignificant (standard deviation = 0.47%). The accuracy revealed a mean percentage recovery of 100.37% with a standard deviation of 0.14%. The method was robust for wavelength variation, instrument and temperature parameters. The limits of detection and quantification of aztreonam were 0.42 and 1.28 µg/mL, respectively. The validated method is linear, precise, accurate, selective, and robust being able to quantify the aztreonam in raw material and in pharmaceutical preparations.

Keywords: *Spectrophotometry; Validation; Analytical Method; Quality Control; Green Chemistry*

Introduction

Aztreonam, the first agent of the monobactam family to be therapeutically approved, was introduced in 1978 from a strain of *Chromobacterium violaceum* in New Jersey and used to treat infections against Gram negative bacteria [1]. It is active against enterobacteria such as *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Serratia marcescens, Salmonella* spp, *Enterobacter* spp., and against *Pseudomonas aeruginosa, Haemophilus influenzae* and *Neisseria meningitides* [2-4]. However, it is ineffective against pathogen Gram positive and anaerobic organisms [5,6]. Chemically, aztreonam is 2-[[[1-(2-amino-4-thiazolyl) -2-[(2-methyl-4-oxo-1sulfo-3-azetidinyl)amino]-2-oxoethylidene] amino]oxy]2-methyl [7,8]. It presents a 1-sulfonic acid attached to the nitrogen on the β-lactam ring, which is responsible for its activation and subsequent reaction with transpeptidase enzymes. The carbonyl group can increase the antibacterial activity and improves stability against *Pseudomonas aeruginosa*. Furthermore, aztreonam contains a 2-aminothiazole side chain that contributes to the excellent activity of the agent against Gram-negative bacteria. Finally, introducing the alpha-methyl group in position 4 stabilizes aztreonam against hydrolysis by the β-lactamase enzymes [9,10]. It is registered in the Chemical Abstract Service (CAS) with the number 01003 and DCB 78110-38-0, its molecular formula is C13H17N5O8S, the molecular weight 435.43 g/mol and has a pH between 4.5 and 7.5 [8]. The chemical structure of aztreonam is shown in Figure 1.

Quantitative spectrophotometric analysis in the ultraviolet region has as its principle the direct relationship between the amount of light absorbed and the concentration of the substance, also known Lambert-Beer law, which is the mathematical basis for measuring radiation absorption at a given wavelength when undergoing a sample since there is an exponential relationship between the light transmission and thickness of absorbent means [12,13].

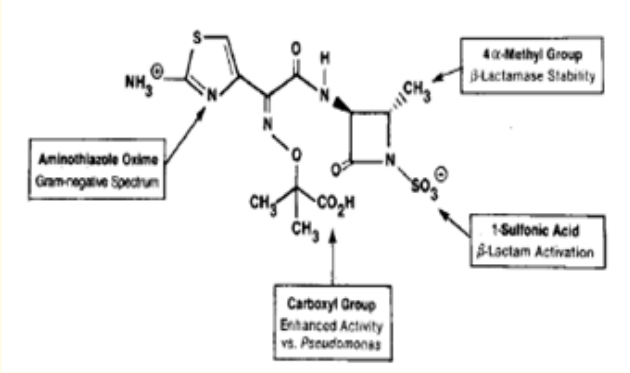

Figure 1: *Chemical structure of aztreonam [11].*

The spectrophotometry in the ultraviolet region occurs by absorption of energy that, when quantized, leads to the passage of electrons from ground state orbital to orbital in a higher-energy excited state, and thus depends on the electronic structure of the molecule, the solvent, the temperature and the length of the wave radiation [11].

The literature has reported analytical methods for the determination of antibiotics such as chromatography, spectroscopy, spectrophotometry and microbiological assay. The microbiological assay is the most commonly used routine method. However, it is slow, often inaccurate, and subject to interference by other antibiotics [14]. Though modern methods of analysis (HPLC, GC, NMR and EM) for purity assays of any drug provide simplicity, speed, specificity, excellent precision and accuracy, they often require sophisticated equipments, which are not in the reach a part of most laboratories and small-scale industries. Moreover, they pose problems of maintenance [15]. On the other hand, spectrophotometry has always provided instrumental simplicity, speed, moderate cost, and portability, and requires less operational training [16,17] and can be perfectly adapted to the routine of a laboratory quality control in the analysis of raw material and the finished product. These features make spectrometric techniques particularly suitable for the determination of low concentrations of clinically important compounds [18,19].

Several methods described in the literature for the quantitative determination of aztreonam use biological samples and pharmaceutical products. These include high performance liquid chromatography (HPLC) with UV detection [20] and UV spectroscopy [21,22]. Unfortunately, they use toxic solvents. In this context, the objective of this study is to develop, validate and apply an analytical method which is cheap, useful and simple to quantify aztreonam with, using spectrophotometry in the UV region to improve quality control and ensure an effective therapy.

Experimental

Sample

The reference substance aztreonam (lot # 0908120) was kindly donated by União Química Pharmaceutical industry (Pouso Alegre, Brazil). Batches of Uni-Aztrenam®, containing 1 g aztreonam lyophilized powder were obtained from commercial sources within its shelf-life.

Instrumentation

All quantitative analyzes and determination of aztreonam were performed a on Shimadzu UV spectrophotometer (model UV mini-1240), using quartz cells of 1 cm optical path, the sonication apparatus Sonicator-Ultrasonic Liquidprocessor, Heat SystemSN (model XL 2020) and a balance analytical (Mettler H51). For all analyzes, the purified water system (Milli-Q Gradient A10 - Millipore, Massachusetts, USA) was used. To achieve robustness, it was used the Shimadzu UV spectrophotometer (model UV 1800).

Preparation of solutions

The stock solution was taken created by accurately weighing 20 mg of aztreonam reference substance, and transferred to putting it into a 100 mL volumetric flask and dissolved in purified water and then finally subjected to sonication for 5 minutes. Finally, the total volume was completed with water to give a concentration of 200 µg/mL. Appropriate dilutions of the stock solution were transferred to 10 mL volumetric flasks, and the volume as completed with purified water and the absorbance was measured at 292 nm for the construction of Ringbom curve in order to establish the concentrations of work to which the drug has linearity (Figure 2).

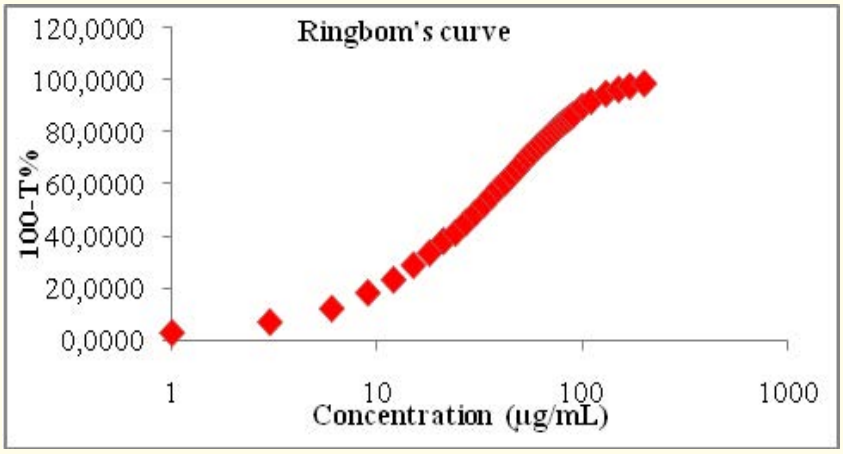

Figure 2: *Ringbom's curve obtained by the UV spectrophotometric method to aztreonam reference substance using purified water as solvent at 292 nm.*

For qualitative analysis aliquots of stock solution were transferred into 10 mL volumetric flask and the volume was completed with different solvents to obtain solutions of 45 µg/mL in purified water (Millipore); hydrochloric acid 0.1 M PA (Synth); sodium hydroxide 0.1 M PA (Merck); methanol PA (Synth); 1% phosphate buffer pH 6 and 1% phosphate buffer pH 8. All solvents used were of analytical grade, the absorption spectra were recorded in the wavelength range between 200 and 400 nm, the same was carried out for aztreonam lyophilized powder and the spectra were compared. For the development of the UV spectrophotometer method, the Ringbom curve was constructed to determine the range in which the drug has a increased linearity.

Method validation

The validation parameters for linearity, selectivity, precision, accuracy, robustness, limit of detection (LD) and limit of quantification (LQ) was according to the parameters established in official guidelines such as Brazil, 2003; FDA, 2004; ICH, 2005; INMETRO, 2011 [23-26].

Linearity

Linearity was established by averaging 3 original standard curves which were obtained at six different levels of concentrations of aztreonam: 27, 36, 45, 54, 63, 72 µg/mL, which were determined in triplicate for each standard curve. The linearity was evaluated by linear regression analysis of standard solutions, using data adjustment by using the least squares method. Furthermore, the data was validated by analysis of variance (ANOVA).

Specificity

Specificity was established by analyzing the excipient (L-arginine) present in the aztreonam samples, evaluated by regression analysis in six concentrations ranging from 27 to 72 µg/mL prepared on three consecutive days (ANOVA). Each concentration was determined in

triplicate. The specificity was also evaluated according to the spectra of solutions of excipient, aztreonam sample and reference substance at a concentration of 200 µg/mL (Figure 3). The excipient solution was prepared under the same conditions as the commercial samples.

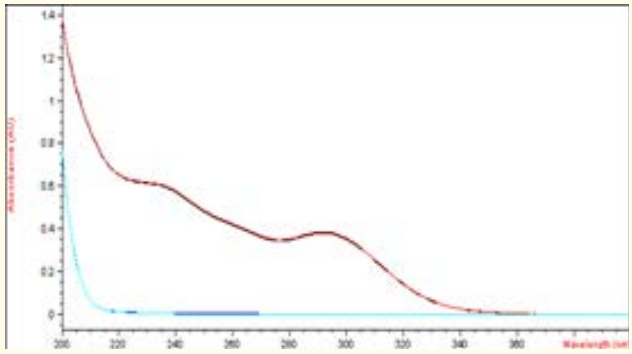

Figure 3: *Absorption spectra in the UV region of aztreonam chemical reference (red), sample (black) and excipient (blue) at a concentration of 200 µg/mL.*

Precision

To study the precision a concentration of 45 µg/mL aztreonam (test concentration) was used. Precision was evaluated in relation to repeatability and intermediate precision. Repeatability, performing six replicates, was assessed by analysis of aztreonam SR solution in the same concentration and the same day. Intermediate precision was studied by performing the analysis on 3 different days and by another analyst in the same laboratory under the same experimental conditions. Seven replications were prepared and analyzed at concentration of 45 µg/mL. All results from the relative standard deviation (RSD) were evaluated.

Accuracy

The accuracy of the method was determined by recovering the standard solution and sample in triplicate on three levels 80, 100 and 120% concentration method (45 µg/mL), according to the ICH recommendations [20]. The aztreonam standard and sample solutions were prepared using purified water. Aliquots of 0.30, 0.95 and 1.40 mL of these solutions (concentrations 36, 45 and 54 µg/mL, respectively) were each added to 1.3 mL solutions of sample in 10 mL volumetric flasks. The flasks were made up to volume with water. The recoveries of aztreonam for testing and relative standard deviation (RSD) were determined.

Robustness

The robustness of the method was checked by the deliberate variations in wavelength, temperature and equipment. The concentration of 45 µg/mL of aztreonam was determined by seven different assays. These results were validated by ANOVA.

Limit of Detection (LOD) and limit of quantification (LOQ)

The limitss of detection (a) and quantification (b) were mathematically determined from the equations:

$$Cs = As \times \frac{Crs}{Ars} \qquad (1)$$

$$Cs\% = Cs \times \frac{100}{Ct} \qquad (2)$$

Where S.D. is the intersection standard deviation and α is the average slope, obtained from the analytical curves of the linearity study.

Results and Discussion

Method development

The methods described in the literature for the determination of aztreonam are time consuming, complex, use toxic solvents and require the use of large amounts of organic solvents and leading to even higher cost. In this green method, a non-toxic solvent was chosen in order to get simple, cheap, and environmentally friendly UV spectrophotometric method for the quantification of aztreonam powder for injection. The spectra of aztreonam reference substance and lyophilized powder at a concentration of 45 µg/mL in many solvents were analyzed, among these solvents, water solvent came up with results similar to phosphate buffer solutions 1% pH 6 phosphate buffer and 1% pH 8, with a maximum absorption wavelength at 292 nm and absorbance around 0.3877 while using methanol as solvent the maximum absorption observed was 0.3412 at 294 nm in an acid medium it was of 284 nm with absorbance of 0.4931 and basic medium it was 268 nm and absorbance of 0.5239. The spectral profiles of aztreonam reference substance and lyophilized powder is shown in Figure 4.

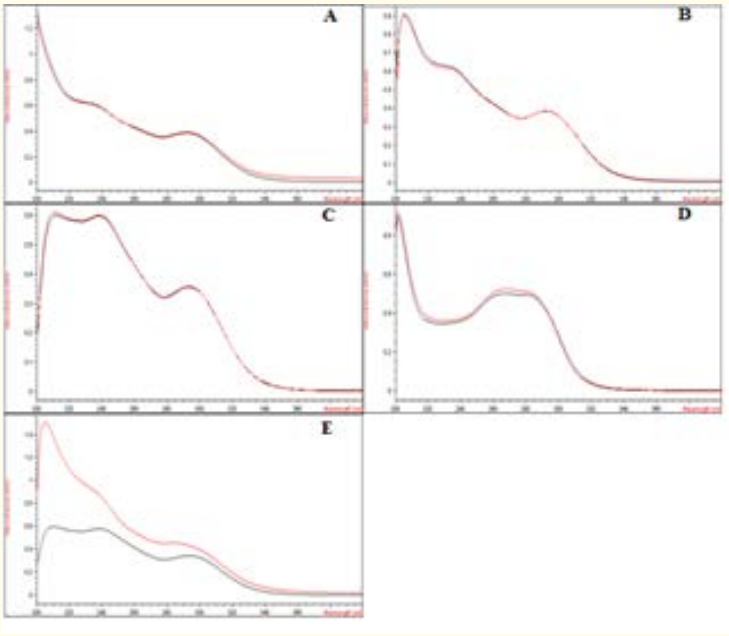

Figure 4: *Absorption spectrum in the ultraviolet region, solution of aztreonam reference substance (black) and lyophilized powder (red) in a concentration of 45 µg/mL, using as solvent: phosphate buffer pH 6 (A); phosphate buffer pH 8 (B); methanol (C); hydrochloric acid 0.1 M (D); sodium hydroxide 0.1 M (F).*

Samples of aztreonam reference substance and lyophilized powder were compared, both kept maximum absorption wavelength with a small difference in absorbance (Figure 3). The results show that the spectral profiles demonstrated for aztreonam reference substance and the sample of lyophilized powder, dissolved in various solvents, are consistent and can be used as proof of identification by comparison. However, in addition to the basic solvent and the reference substance and lyophilized powder behaved differently. This happened due to rapid degradation of aztreonam in basic medium. After analyzing the spectra, purified water was chosen to use as a solvent, by presenting appropriate features in the spectra and economic and environmental advantages, such as being easy acquisition and disposal and low cost. The spectrum scan of aztreonam dissolved in purified water showed maximum absorbance at 292 nm wavelength (Figure 2). Furthermore, there was no interference from the adjuvent L-arginine in the region of the absorption spectrum of aztreonam powder for injection, showing maximum absorbance at a wavelength of 366 nm (Figure 2).

Method Validation

Linearity

The analytical curves, resulting in from three consecutive days were fitted by linear regression analysis, whose straight line equation is: y = 0.0096 x + 0.0026. The coefficient correlation was 0.9998 (Figure 5). Furthermore, data was validated by an analysis of variance (ANOVA), which showed highly significant regression, since the F values calculated were smaller than those critical values of F (Table 1).

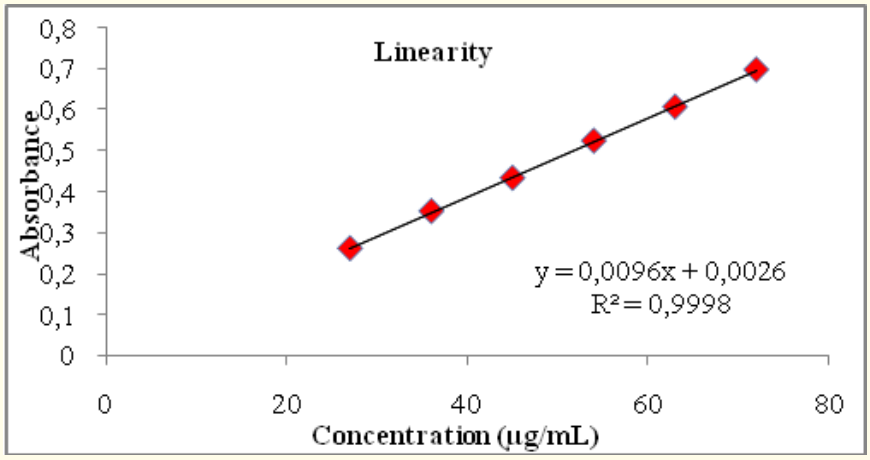

Figure 5: *Calibration curve of aztreonam reference substance obtained by the spectrophotometric method in the UV region, using water as solvent at 292 nm.*

Source of variation	DF	Sum of square	Variability	F calculated	F critical
Between concentration	5	0.39381	0.07876	1060.11*	3.11
Linear regression	1	0.39378	0.39378	5300.16*	4.75
Deviation of linearity	4	0.00003	0.0000075	0.10	3.26
Residue	12	0.0008915	0.000074296	-	-
Total	17	0.39470	-	-	-

Table 1: *Analysis of variance of absorbance values determined in the obtaining of the calibration curve of aztreonam reference substance using the spectrophotometric method in the UV region.*

** Significant at p < 0.05%*

Specificity

Data from the calibration curve resulting from the average of three sample curves generated on three consecutive days, whose straight line equation is: y = 0.0098 x + 0.0016. The correlation coefficient was 0.9999, meaning that 99.99% of the total variation around the mean, with residual (error) of only 0.01% (Figure 6). Data was validated by ANOVA, where the calculated F values were smaller than those F critical values (Table 2). Furthermore, specificity was confirmed by spectral analysis showed that the adjuvant has no absorbance at the wavelength used in this method (Figure 2).

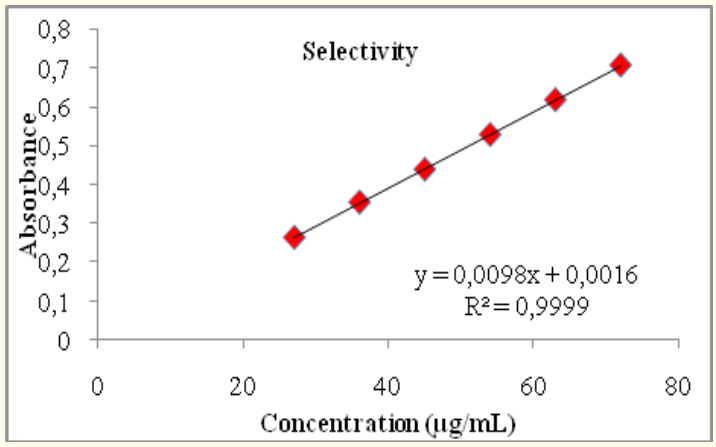

Figure 6: *Calibration curve of aztreonam reference substance obtained by the spectrophotometric method in the UV region, using water as solvent at 292 nm.*

Source of variation	DF	Sum of square	Variability	F calculated	F critical
Between concentration	5	0.40753	0.08151	4588.56*	3.11
Linear regression	1	0.40750	0.40750	22941.1*	4.75
Deviation of linearity	4	0.00003	0.0000075	0.42	3.26
Residue	12	0.0002132	0.000017763	-	-
Total	17	0.40774	-	-	-

Table 2: *Analysis of variance of absorbance values determined in the obtaining of the calibration curve of aztreonam reference substance using the spectrophotometric method in the UV region.*

** Significant at p < 0.05%*

Precision

Precision was evaluated according to repeatability and intermediate precision. Repeatability was determined by calculating the relative standard deviation (RSD) for seven repetitions of 45 µg/mL test concentration under the same experimental conditions on the same day. The RSD value obtained was 0.20%. The intermediate precision was evaluated by calculating the recovery of the drug performed on 3 different days (inter-day precision). The RSD value obtained was 0.42%. For the inter-analysts precision between the RSD was 0.47% (Table 3). Values less than 5% confirm the method is precise. The interday precision was evaluated by analysis of variance while the between-analyst precision was evaluated by Student's t test, as shown in Tables 4 and 5.

Analytical responses				
		Content (µg)[a]	Content (%)[a]	RSD (%)
Repeatability	0.4528[a]	20.00	-	0.2085
	Day 1	20.24	99.35	
Intermediate precision	Day 2	20.53	100.79	0.4260
	Day3	20.39	100.13	
	Analyst 1	20.23	101.17	0.4728
	Analyst 2	20.37	101.85	

Table 3: *Method Precision Results for aztreonam.*

[a] mean of seven replicates

Source of variation	DF	Sum of square	Average squares	F calculated	F critical
Between groups	2	4.64857E-06	2.3243E-06	0.839236	3.55
Within groups	18	4.98514E-05	2.7695E-06	-	-
Total	20	5.45E-05	-	-	-

Table 4: *Data obtained from analysis of interday precision spectrophotometric method in the UV region for determination of aztreonam, by analysis of variance (ANOVA).*

** p < 0.05%*

Source of variation	Average	Degree of freedom	Variance	T calculated	T critical
Analyst 1	0.4445	7	6.15619E-06	-9.98	2.17
Analyst 2	0.4556	7	2.47238E-06		

Table 5: *Data obtained from analysis of the accuracy of analyst spectrophotometric method in the UV region for analysis aztreonam, by Student's t test.*

Accuracy

The accuracy of the method was confirmed by determining the average recoveries of samples using the standard addition method. As shown in Table 2, the mean percentage recovery was 100.37%, with a standard deviation was of 0.14%. The results demonstrate that slight variations in the concentration of aztreonam can be readily quantified by the method as well as no interference of excipients therefore the analytical method developed is sufficiently accurate.

Sample 200 µg/mL	Reference standard concentration (µg/mL)		Recovery (%)	RSD (%)	Mean recovery (%)
	Added	Found			
	36	36.47	101.3	0.30	
	45	45.45	101.0	0.10	100.9
	54	54.22	100.4	0.20	

Table 6: *Method Accuracy Results for aztreonam.*

Robustness

The robustness was found to be reliable, as determined by the R.S.D. (< 5%). The experimental parameters wavelength, temperature and instrument were tested on their influence on the consistency of the method. The RSD using varying wavelength was 0.83%, for the temperature change 0.02% and for changing the instrument 0.17%. Furthermore, data was validated by variance analysis, and the F values calculated were smaller than those critical values of F, showing no statistically significant difference existing between the means, the method is robust. Small changes that occurred during the analyzes did not affect the absorption intensity of the samples.

Limits of detection (LOD) and quantitation (LOQ)

LOD and LOQ values were found to be 1.28 µg/mL and 0.42 µg/mL, respectively. The method is sensitive to small concentrations.

Assay of the pharmaceutical product

The validated method was applied to the determination of aztreonam for injection. The results, expressed as the percentage drug is in accordance with the Brazilian Pharmacopoeia [9]. Spectrophotometry in the UV-VIS is a widely used technique to quantify antibiotics and

other drugs [27-33], because they are inexpensive, simple and do not require complex sample preparation [34]. Moreover, they produce very low amounts of residues and require only small amounts of solvents, which is an important ecological important aspect [35-51].

The applicability of the method to test pharmaceutical preparations was examined. The results are highly reproducible for determination of the aztreonam.

Conclusion

Though green chemistry is still challenging, we have come a long way to be able to optimize analytical methods using green solvents and miniaturized techniques.

The validated analytical method for the quantitative determination of aztreonam has the advantages of simplicity, speed, low-cost conditions, a lack of polluting reagents and generates only a small amount of residue. All validation parameters were found to be highly satisfactory, including linearity, accuracy, precision, selectivity, robustness and adequate detection and quantitation limits. The validated method is a good alternative for quality control of aztreonam by the pharmaceutical industry and quality control laboratories.

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Declaration of Interest

The authors report no declarations of interest.

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