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Spectrophotometric Determination of Aztreonam in Bulk and its Pharmaceutical Formulations

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Three simple and sensitive methods for the assay of aztreonam were developed. Methods A-C are based on the oxidation of aztreonam with an excess of oxidant [N-bromo succinamide (NBS) in methods A and B or chloramine T (CAT) in method C] in acidic medium. The unreacted oxidant is then estimated by using a oxidisable dye [celestine blue (CB) in method A or gallocyanine (GC) in method C] or *p*-N-methyl amino phenyl sulphate (PMAP)-sulphanilamide (SA) reagent in method B. Regression analysis of Beer's law plots showed good correlation in the concentration ragne of 4-20, 4-20 and 4-20 μg/mL for method A, B and C, respectivley. The results of analysis have been validated statically and by recovery studies.

Key Words: Aztreonam, Charge-transfer-complexation.

INTRODUCTION

Aztreonam (AZA)[1] is a synthetic bactericidal antiboitic and originally isolated from chromobacterium violaceum. Aztreonam is designated chemically as (Z)-2-[[[(2-amino-4-thiazolyl)[[(2S,-3S)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]-carbamoyl]-methylene]amino]oxy]-2-methylpropionic acid. Few physico-chemical methods appeared in the literature for the determination of aztreonam in pharmaceutical formulations. The methods so far reported include, biological assay[2], HPLC[3-8] and spectrophotometer[9-14].

The reported spectrophotometric methods possesses dificiencies such as low λ_{max} or low sensitivity and the analytically important functional groups of aztreonam do not seem to have been fully exploited for designing suitable spectrophotometric methods for its determination. The author has made some attempts in this direction following the five reactions (NBS-CB, method A; NBS-PMAP, method B; CAT-GC; method C) for the determination of aztreonam utilizing its structural futures. All the methods are applicable to the determination of AZA in bulk form and in formulations.

EXPERIMENTAL

A Milton Roy UV-vis spectrophotometer 106 with 1 cm matched quartz cells were used for all spectral and absorbance measurements. A Elico LI-120 digital pH meter was used for pH measurements.

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All the reagents were Analytical grade. All the solutions were prepared fresh in double distilled water. Aqueous solutions of N-bromo succinamide (NBS) (Loba, 5.618×10^{-4} M) and HCl (E-Merek, 5 M) were prepared for method A. Aqueous solutions of NBS (Loba, 5.618×10^{-3} M), *p*-N-methyl amino phenyl sulphate (PMAP) (Loba, 8.71×10^{-3} M) were prepared for method B. Aqueous solutions of chloramine T (CAT) (Loba, 7.10×10^{-4} M), gallocyanine (GC) (Chroma, 2.9698×10^{-4} M) were prepared for method C.

Preparation of drug solutions: The stock solution (1 mg/mL) of AZA was prepared by dissolving 100 mg of it initially in 10 mL of 0.1 M HCl, followed by dilution to 100 mL with same solvent. This soluiton was further diluted step wise with distilled water to obtain working standard solution of concentrations of 200 µg/mL (method A-C).

Method A: Aliquots of drug solution (AZA; 0.5-2.5 mL; 200 µg mL^{-1}) were taken in series 25 mL calibrated tubes, 1.25 mL of (5.0 M) HCl, 2.5 mL of (5.618×10^{-4} M) NBS solution were added and the volume was made upto 20 mL with distilled water. After 10 min, 5 mL of (5.49×10^{-4} M) celestine blue (CB) was added and mixed throughly. The absorbances were measured after 5 min at 520 nm against distilled water. The blank (omitting drug) and due (omitting drug and oxidant) solutions were prepared in a similar manner and their absorbances against distilled water. The decrease in a absorbance corresponds to the consumed NBS and in turn to the drug concentration, was obtained by subtracting the decrease in absorbance of test solution (dye-test) from that of the blank solution (eye-blank). The amount of drug in sample was obtained from its Beer's law plot of drug concerned.

Method B: Aliquots of the standard drug solution (AZA; 0.5-2.5 mL; 200 µg mL^{-1}) were transferred into a series of 25 mL-calibrated tubes containing 0.5 mL of (8.75×10^{-1} M) AcOH and 2 mL of (5.618×10^{-4} M) NBS solution. The volume was made upto 10 mL with distilled water and kept aside for 15 min. Then 1.5 mL of (8.71×10^{-3} M) PMAP solution and after 2 min, 2 mL of (1.16×10^{-2} M) sulphanilamide (SA) was added. The volume was made upto 25 mL with distilled water and the absorbance was measured after 10 min, at 530 nm against distilled water. A blank experiment was also performed omitting drug solution. The decrease in absorbance corresponding to drug content was obtained by subtracting the absorbance of the test solution from that of blank solution. The amount of drug present was calculated from its calibration curve.

Method C: Aliquots of the standard drug solution (AZA; 0.5-2.5 mL; 200 µg mL^{-1}), 1.25 mL of (5 M) HCl and 2 mL (7.1×10^{-4} M) CAT were taken into a series of 25 mL calibrated tubes and the volume was made upto 20.0 mL with distilled water. After 10 min, 5 mL (5.938×10^{-4} M) gallocyanine was added and mixed thoroughly. The absorbances were measured after 5 min at 540 nm for AZA against distilled water. The decrease in absorbance corresponding to consumed CAT and in turn to drug content, was obtained by subtracting the decrease in absorbance of test solution (dye-test) from that of the blank solution (dye-blank). The amount of drug in sample was obtained from its Beer's law plot of the drug concerned.

RESULTS AND DISCUSSION

The optimum conditions for each method were established by varying one parameter at a time and keeping the others fixed and observing the effect produced on the absorbance of the coloured species.

The optical characteristics such as Beer's law limits, molar absorptivity for each method are given in Table-1. The precision of each method was tested by estimating 6 replicate samples of the drug with in the Beer's law limits and the results obtained are incorporated in Table-1. Regression analysis using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each method and are presented in Table-1. The accuracy of each method was ascertained by comparing the results by proposed and reference method (UV) statistically (Table-2). The comparision shows that there is no significant difference between the results of propsed methods. The similarity of the results is obviously evident that during the application of the methods, the additives and excipients present in tablets do not interfere in the assay of proposed methods. As an additional check of accuracy of the proposed methods, adding a fixed amount of the drug to the preanalyzed formulations performed recovery experiments. The amount of drug found and the % recovery was calculated in the usual way.

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY
OF THE PROPOSED METHOD FOR AZTREONAM

Optical characteristics	Method A NBS/CB	Method B NBS/PMAP-SA	Method C CAT/GC
λ_{max} (nm)	590	530	540
Beer's law limits (µg/mL)	4-20	4-20	4-20
Molar absorptivity (λ mol^{-1} cm^{-1})	1.692×10^4	1.344×10^4	1.464×10^4
Correlation coefficient (r) Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.0260	0.0320	0.0300
Regression equation (y = a + bc) (i) slope (b)	0.0389	0.0311	0.0340
(ii) Standard deviation on slope (S_b)	1.7×10^{-4}	3.2×10^{-4}	3.4×10^{-3}
(ii) Intercept (a)	-0.00170	0.0003	-0.0061
(iv) Standard deviation on intercept (S_a)	2.26×10^{-3}	4.21×10^{-3}	4.45×10^{-3}
(v) Standard error of estimation (S_e)	2.15×10^{-3}	4.01×10^{-3}	4.24×10^{-3}
Relative standard deviation* % of range error (confidence limit)	0.27760	0.2593	0.3367
(i) 0.05 level	0.23200	0.2170	0.2820
(ii) 0.01 level	0.34300	0.3210	0.4170
% Error in bulk sample**	-0.02000	-0.0510	0.2090

Conclusion

The proposed methods are applicable for the assay of drug (AZA) and have the advantage of wider range under Beer's law limits. The decreasing order of sensitivity and λ_{max} among the proposed methods are A > C > B and A > C > B, respectively. The proposed methods are simple, selective and can be used in the routine determination of AZA in bulk samples and formulations with reasonable precision and accuracy.

TABLE-2
DETERMINATION OF AZTREONAM IN PHARMACEUTICAL FORMUATIONS

Sample	Labelled amount (mg)	Amount found by proposed methods			Ref. method	Recovery by proposed methods (%)		
		M_7	M_8	M_9		M_7	M_8	M_9
Inj. I	250	249.40 ± 1.060 F = 2.31 t = 0.79	248.5 ± 0.99 F = 2.93 t = 0.98	250.60 ± 1.280 F = 3.36 t = 0.49	250.10 ± 0.698	99.80 ± 0.42	99.40 ± 0.39	100.30 ± 0.51
Inj. II	250	240.10 ± 2.102 F = 1.40 t = 0.43	249.7 ± 0.560 F = 2.14 t = 0.65	248.80 ± 1.921 F = 1.17 t = 0.65	249.60 ± 1.776	99.60 ± 0.84	99.90 ± 0.22	99.50 ± 0.77
Inj. III	500	499.22 ± 1.340 F = 2.76 t = 0.10	498.45 ± 1.770 F = 2.15 t = 0.99	499.18 ± 0.720 F = 1.34 t = 1.86	496.76 ± 1.864	99.80 ± 0.33	99.61 ± 0.44	99.79 ± 0.18
Inj. IV	500	499.48 ± 0.890 F = 2.28 t = 1.73	498.26 ± 1.730 F = 1.03 t = 0.10	499.89 ± 1.950 F = 1.83 t = 1.86	496.93 ± 0.695	99.87 ± 0.22	99.56 ± 0.43	99.97 ± 0.48

Average (± RSD) of 6 determinations; that t and F values refer to comparison of the proposed method with the reference method; theoretical values at 95 % confidence limits, t = 2.57, F = 5.05.

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