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METHOD DEVELOPMENT AND VALIDATION OF AZTREONAM BY RP-HPLC IN BULK DRUG AND FORMULATION USING PARACETAMOL AS INTERNAL STANDARD

Vipin Prakash*[1],
Sharif niroush konari[1], Gerad suresh[2]

[1]JDT Islam College of Pharmacy, Calicut -673 012,kerala.
[2]College of Pharmacy, Madras medical college, Chennai.

ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the estimation of Aztreonam using Paracetamol as internal standard. A waters spherisorb ODS 5µm column (150 x 4.6 mm) in isocratic mode, with mobile phase containing methanol: buffer (10:90 %v/v) pH 2.6 adjusted with orthophosphoric acid were used. The flow rate was 1ml/min. The retention time of Aztreonam and Paracetamol were 10.6 and 4.9 min respectively, and the resolution factor was 3.93. Linearity range for Aztreonam was 5- 25 µg/ml. The proposed method is accurate, precise, specific and rapid for the estimation of Aztreonam injection.

Key words: Aztreonam, RP- HPLC, Paracetamol, Method development.

Correspondence to Author

Vipin Prakash

Assistant Professor JDT Islam College of Pharmacy Calicut-673 012, Kerala.

Email: 1vipin1@gmail.com

INTRODUCTION

Aztreonam[1-2] is a synthetic monobactam bactericidal antibiotic originally isolated from chromo bacterium violaceum. It is a white crystalline powder. Aztreonam is chemically (z)-2-[[[(2- amino - 4-thiazolyl) [[(2S,- 3S) - 2- methyl-4- oxo -1- sulfo -3-azetidinyl] carbomoyl] methylene] amino] oxy] -2- methyl propionic acid ,which is used in the treatment of life threatening infections with susceptible gram-negative aerobic organisms, especially Pseudomonas aeruginosa. Aztreonam acts by inhibiting bacterial cell wall peptidoglycan synthesis. Literature reviews [3-11] revealed, no method of estimation for aztreonam using paracetamol as internal standard in bulk and formulation by high performance liquid chromatography [12-15] has been reported so far except in biological fluids. All the measurements were made using Shimadzu HPLC. All the solutions were freshly prepared by using HPLC grade solvents.

MATERIALS AND METHOD

Preparation of mobile phase and standard stock solution : Mobile phase was prepared by mixing Methanol and buffer (potassium dihydrogen ortho

phosphate) in proportion of 10:90 %v/v, pH 2.6 adjusted with orthophosphoric acid. The standard stock solutions of Aztreonam and paracetamol (100µg/ml)were prepared separately by dissolving 10mg of drug in 100 ml of water.

Calibration curve

Aliquots of 0.5,1,1.5,2,2.5 ml from standard Aztreonam and 1ml of standard Paracetamol were transferred to different volumetric flasks of 10ml.The volume was adjusted to the mark with water gave a solution containing 5,10,15,20 and 25µg/ml Aztreonam and 10mcg/ml of Pracetamol (internal standard).

The mixed standard solution was chromatographed for 20 mins using mobile phase at a flow rate of 1ml/min. Plot the graph of peak area vs concentration for both the drugs. Temperature of the column was kept at ambient, and the effluent was monitored at 298 nm. The retention time of AZT is 10.6 min and PARA is 4.9 min respectively with resolution factor of 3.93.Chromatogram shown in Fig.1

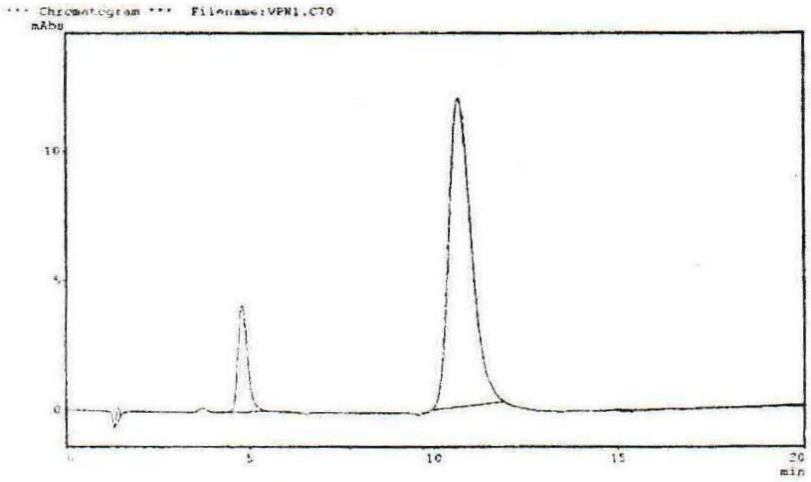


Fig.1

Analysis of formulation

A vial of AZT injection was dissolved in 10 ml water for injection.From this dilution is made to a concentration of 100mcg/ml.1ml of this solution is transferred in to a 10 ml standard flask and added 1ml of Paracetamol stock solution (100 µg/ml) and the volume is made upto 10 ml with water to give a solution containing 10mcg/ml AZT and 10 µg/ml PARA. This solution was used for the estimation of AZT. The prepared sample solution was chromatographed for 20 mins using mobile phase at a flow rate of 1ml/min (Fig.2).From the peak area obtained in the chromatogram, the amount of AZT was calculated (table.4) and the chromatogram shown in figure.2.

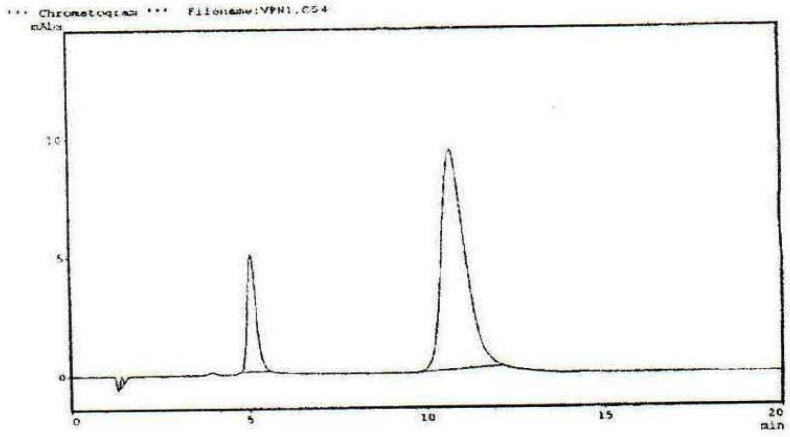


Fig.2

RESULTS AND DISCUSSION

Several mobile phases with different ratios and pH were tried and no symmetrical peaks were obtained except with methanol: potassium dihydrogen ortho phosphate buffer. So this mobile phase was selected for the study. After mixing the mobile phase, effect of ionic strength of phosphate buffer, ratio of mobile phase, pH and flow rates were studied. From these, strength of potassium dihydrogen ortho phosphate was fixed as 25mM, ratio of mobile phase as 10:90 % v/v,pH 2.6 and a flow rate of 1ml/min as optimum parameters for this study. Under this condition, Aztreonam showed a good symmetrical peak with the retention of 10.6 mins. Several drugs were tried for the selection of internal standard and Paracetamol was selected because it gave a symmetrical peak in same mobile phase of Aztreonam. The whole content(500mg) of the vial (Aztreonam formulation) was diluted with water(HPLC grade) to get a concentration ranging from 5 – 25 µg ml. This solution was also contained 10 mcg/ml of internal standard. The internal standard and Aztreonam were eluted at 4.9 and 10.6 mins respectively. The calibration curve so obtained showed linearity range between 5- 25 µg/ml with the correlation coefficient of 0.9998(Fig.3).The LOD and LOQ of Aztreonam were found to be 20 ng /ml and 5 mcg/ml respectively (Fig .4).To study the validity [13-18] of the method, recovery and repeatability studies were carried out using the same optimum conditions(table.2 & 3).The system suitability studies [19-23] were also calculated which includes column efficiency,resolution,capacity factor, selectivity factor and peak asymmetry factor (table.1).The proposed method was found to be accurate ,simple ,sensitive and rapid. The low standard deviation value and good percentage recovery indicate the reproducibility and accuracy of the newly developed methods.

Table 1: System suitability paramete

Parameter	AZT
Retention time	10.6
Resolution	3.93
Theoretical plate	12,501
Capacity factor	3.2
Peak Assymetry factor	1

Table 2: Validation parameters

Parameter	AZT
Calibration range	5-25 µg/ml
Accuracy	102.3 ± 0.19
Precision (%RSD)	
Intra-day (n=3)	0.006
Inter-day (n=3)	0.0193
Repeatability (% RSD)	0.005
Correlation coefficient	0.9998
LOD	20 ng/ml
LOQ	5 µg/ml

Table 3: Recovery data

Drug	Label claim (mg/vial)	Amount present * (mg/vial)	% recovery ±S.D*	
			50%	100%
AZT	500	501.52	102.1 ±0.25	102.3 ± 0.19

Table 4: result of analysis of formulation

Drug	Amount (mg/vial)		% label claim ± S.D*	R.S.D*
	Labelled	Found*		
AZT	500	501.52	100.3 ±0.038	0.0078

*Average of 3 determinations.

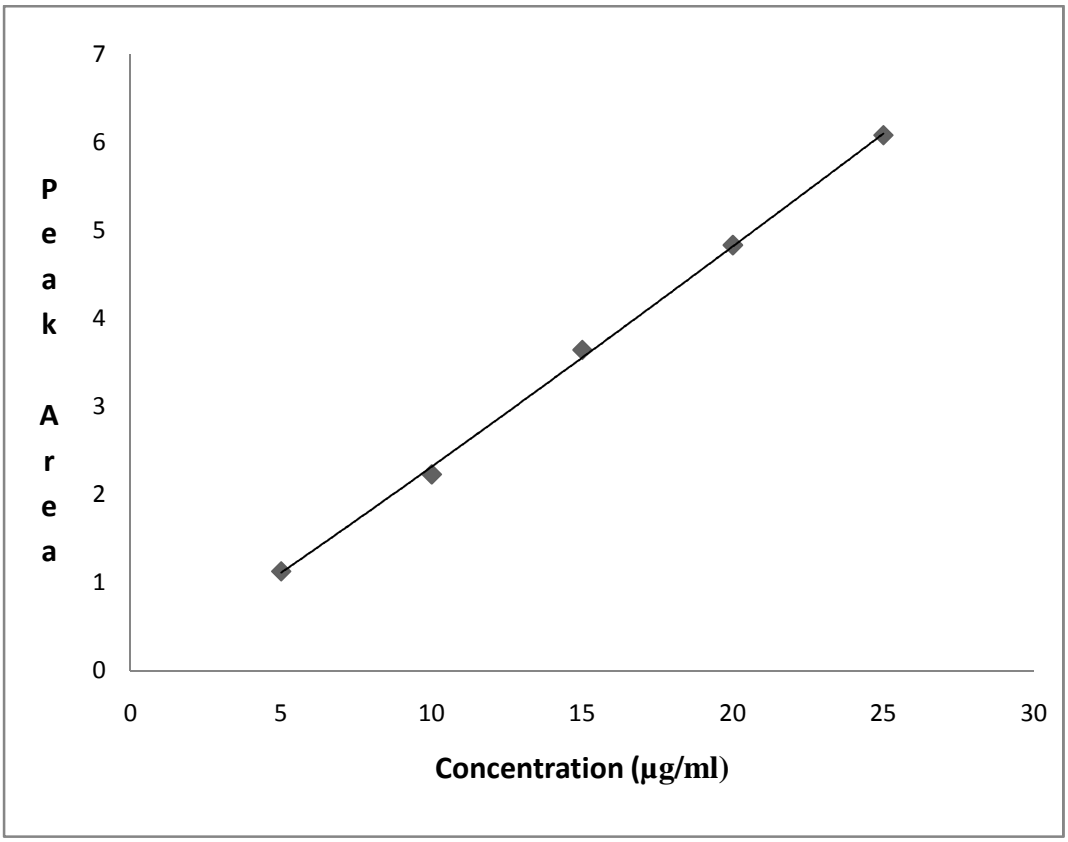
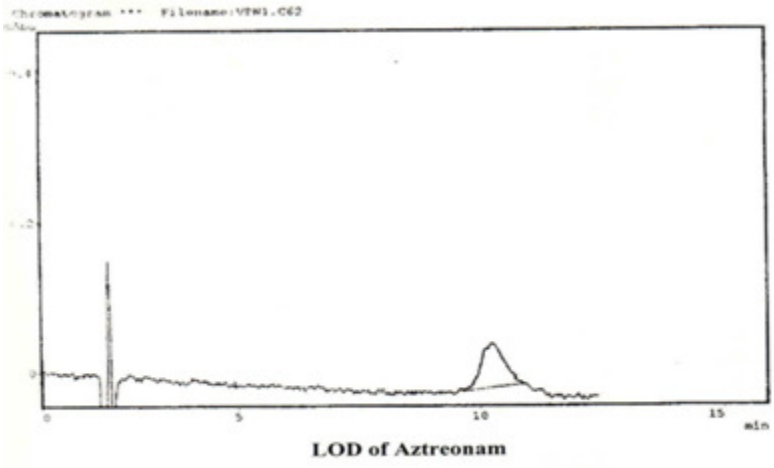
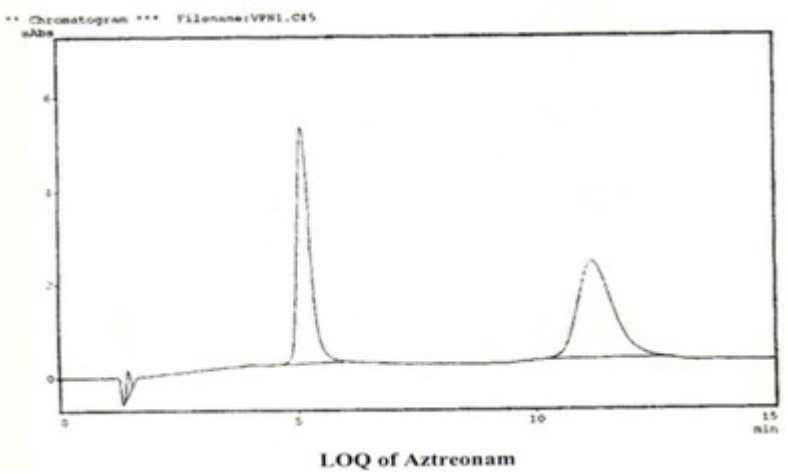


Fig.3



LOD of Aztreonam

Fig.4(A)



LOQ of Aztreonam

Fig.4(B)

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