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Research Article

RP-UFLC-DAD METHOD FOR THE DETERMINATION OF PIPERACILLIN AND TAZOBACTAM SODIUM IN BULK AND MARKETED FORMULATIONS

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ABSTRACT

Aim: To develop a simple, sensitive, rapid, robust and reproducible method for the simultaneous determination of piperacillin and tazobactam sodium using ultra fast chromatographic method (UFLC).

Materials and Methods: The analysis was performed on Phenomenex C_{18} column using acetonitrile, methanol and 1% orthophosphoric acid (pH 2.5) in the ratio of 50:30:20 v/v/v as mobile phase with flow a rate 1ml/min. The eluents were monitored with PDA detector at 225nm. The method was validated as per Q2 (R1) guidelines. The proposed optimised method is having linearity in the concentration range from 5-25 µg/ml of tazobactam sodium and 40-200µg/ml for piperacillin.

Results: In this developed method piperacillin and tazobactam sodium were eluted at a retention time of 4.5 ± 0.1min and 3.2 ± 0.1min respectively. The present method is linearity, accurate, precise and has passed all system suitability parameters.

Conclusion: The proposed method can be readily utilized for determination of piperacillin and tazobactam sodium.

Key words: Piperacillin, Tazobactam, Simultaneous determination, Validation

INTRODUCTION

Piperacillin is a semi-synthetic broad-spectrum antibacterial agent and is indicated for the treatment of serious infections caused by susceptible strains of microorganisms. It is chemically known as [2s-[2α, 5α, 6 β (s*)]]-6[[[[(4-ethyl-2,3-dioxo-1-piperazinyl]carbonyl]amino] phenyl-acetyl]amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0] heptane-2 carboxylic acid.[1] The structure of Piperacillin is shown in figure 1**.**

Tazobactam is a compound that inhibits the action of bacterial β-lactamases. It is chemically known as (2S, 3S, 5R)-3-methyl-oxo-3-(1h-1,2, 3-triazol-1-ylmethyl)-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid 4, 4-dioxide. It is combined with the extended spectrum β-lactam antibiotic Piperacillin in the drug Piperacillin/tazobactam.[2] It is one of the preferred antibiotic treatments for nosocomial pneumonia caused by *pseudomonas aeruginosa*. It broadens the spectrum of Piperacillin by making it effective against organisms that express β-lactamase and would normally degrade Piperacillin. Tazobactam sodium is a derivative of the penicillin nucleus and is a penicillanic acid sulfone.[3] The structure of tazobactam is shown in figure 2. The combination of piperacillin and tazobactam is used to reduce the development of drug-resistant bacteria.

Examination of literature reveals that there are few RP-HPLC methods for the simultaneous determination of Piperacillin and Tazobactam.[4-8] Though these methods are have some demerits like more retention time, complex mobile phases, use of salts and inaccurate methods like HPTLC. The main objective of this work is to develop a simple, rapid and precise method for simultaneous determination of piperacillin and tazobactam using ultra-fast liquid chromatography and to focus on the drawbacks of the above mentioned methods. The method was thoroughly tested for its specificity and selectivity according to the ICH guidelines.

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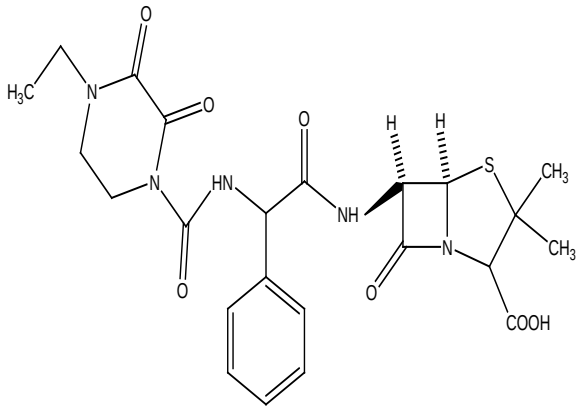

Fig 1: Structure of Piperacillin

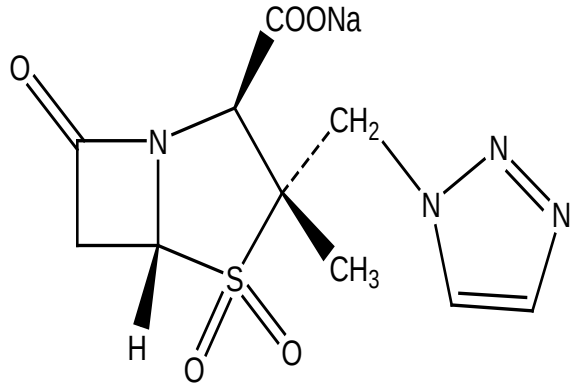

Fig 2:Structure of Tazobactam

MATERIALS AND METHODS

Instrumentation:

Chromatographic separation was carried out on UFLC (SHIMADZU) LC-20AD equipped with a 1260 binary pump VL (35 MPa), Prominence SIL-20ACHT Auto sampler and prominence SPD-M20A Diode array detector connected to a computer loaded with LC solution software. Weighing of the samples was performed on a Shimadzu electronic analytical balance AY-220. Sonication was done by using an ultrasonic bath (Mark ultrasonic sonicator).

Materials:

The chemicals used were of analytical grade and HPLC grade [methanol, acetonitrile and orthophosphoric acid (E.Merk Ltd., Mumbai)]. The pure API of Piperacillin and Tazobactam were obtained as a gift sample from Micro Labs, Bangalore. The combination of piperacillin and tazobactam containing formulations i.e., Tazar, Tazact and Durataz were purchased from the local pharmacy.

Chromatographic conditions

The mobile phase was a mixture of acetonitrile: methanol: 1% Orthophosphoric acid (pH 2.5) in the ratio of 50: 30:20 v/v/v. The separation was done at ambient temperature and the detection wavelength was 225nm. The injection volume was 10ml with a run time of 15 minutes.

Preparation of standard solutions

10mg of Piperacillin and Tazobactam sodium were weighed and taken into separate 10ml volumetric flasks and dissolved in acetonitrile. Volume was made up using the same solvent to obtain standard stock solutions. The above solutions were further diluted to get working standard solutions.

Preparation of test solution

Test solution was prepared from different marketed formulations like Tazar (4.5g), Durataz (4.5g) and Tazact (2.25g). The powder equivalent to 1.6mg of Piperacillin and 0.2mg of Tazobactam was weighed and made up to 10ml with mobile phase after dissolving the sample to obtain a sample solution.

Assay

The sample solution was injected along with the same concentration of standard solution and chromatogram was recorded. The peak area values were calculated. The amount of piperacillin and tazobactam solution was estimated using the calibration curve method. The results were tabulated in Table 6.

Validation [9]

The method was validated for different parameters like system suitability, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness according to ICH Q2 (R1) guidelines.

System suitability

System suitability was assessed by six replicate analysis of the drug at concentration of 40µg/ml. The system suitability parameters like tailing factor (NMT 1.5) and theoretical plate count (NLT 2000) are determined.

Linearity

From the experimental conditions described above, the linear calibration curves of piperacillin

and tazobactam sodium was obtained for five different concentration levels. Linear correlation was found by the regression equation.

Precision

The intra-day and inter-day precision of the assay method was evaluated by carrying out the assay and analyze corresponding response six times on the same day and on different days for the sample solution.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were calculated based on the response and the slope of a regression equation.
LOD = 3.3(SD)/S and LOQ = 10(SD)/S, where SD= standard deviation of response and S= slope of the calibration curve.

Robustness

According to the ICH, robustness for an analytical procedure is a "measure of its capability to remain unaffected by minor, but deliberate variations in method optimized conditions. The characteristic variations studied under this parameter are mobile phase composition (% acetonitrile and methanol), pH, flow rate (ml/min), temperature (^{0}C) and wavelength (nm).

Specificity

Specificity is the capability of the method to measure the analyte response in the presence of its degradation products. The peak purity indices for the analytes in stressed solutions determined with PDA detector under optimized chromatographic conditions were found to be better indicating that no additional peaks were co-eluting with the analytes and evidencing the ability of the method to assess unambiguously the analyte of interest in the presence of possible interference. Baseline resolution was achieved for both the drugs.

RESULTS AND DISCUSSION

Method development and validation

Piperacillin and Tazobactam are strongly acidic in nature. Taking into consideration, the pH value of the mobile phase should be acidic. After some trials, 1% orthophosphoric acid with pH 2.5 was finally selected. A mixture of acetonitrile, methanol and 1% orthophosphoric acid with pH 2.5 (50:30:20) was optimized as mobile phase, which produced symmetric peak shape, good resolution and reasonable retention time for both the drugs. Retention times of piperacillin and tazobactam for 6 repetitions were found to be 4.5 ± 0.1min and 3.2 ± 0.1min respectively. The chromatograms of a standard, sample solution and blank were shown in figure 3, 4, 5 & 6. The wavelength employed for the detection was 225nm. The optimized chromatographic conditions were shown in Table 1**.** The Peak purity of piperacillin and tazobactam sodium were shown in fig 7 & 8.

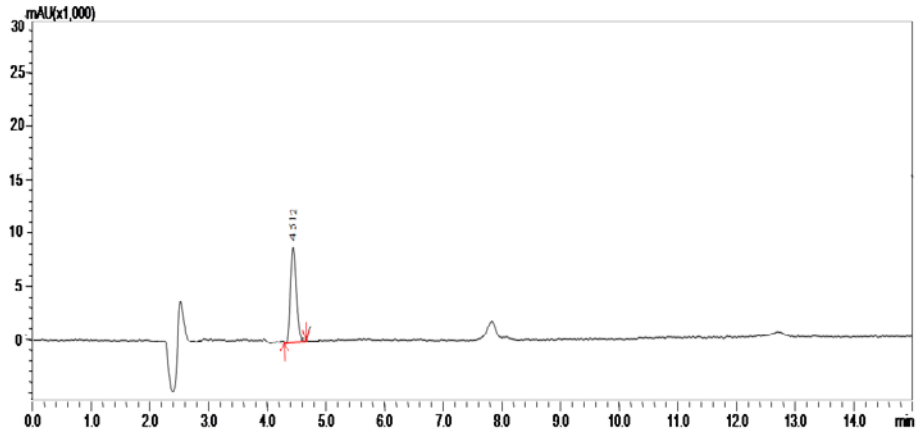

Figure 3: Chromatogram of standard Piperacillin

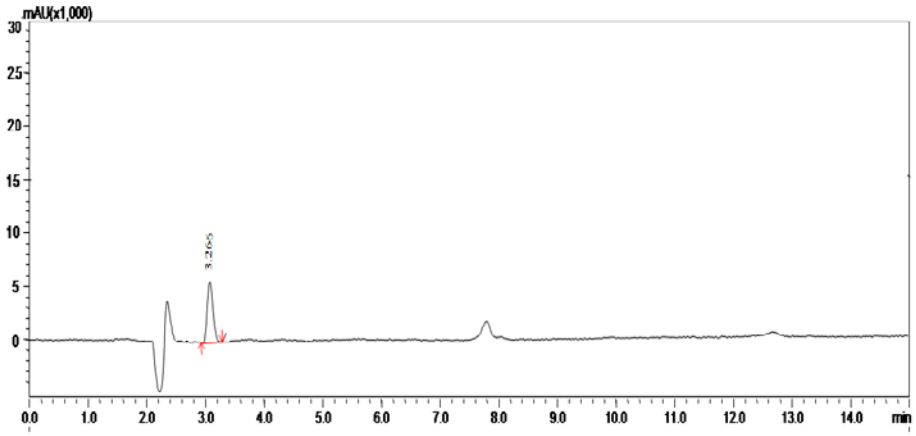

Figure 4: Chromatogram of standard tazobactam sodium

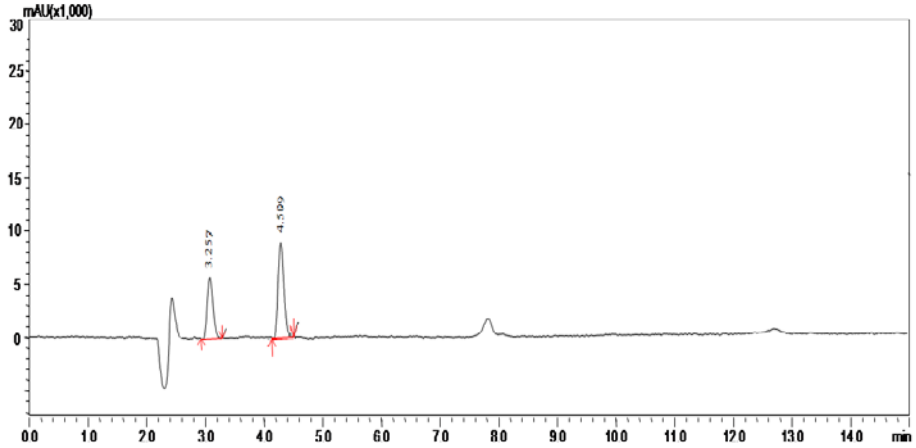

Figure 5: Chromatogram of sample Piperacillin and Tazobactam sodium in formulation

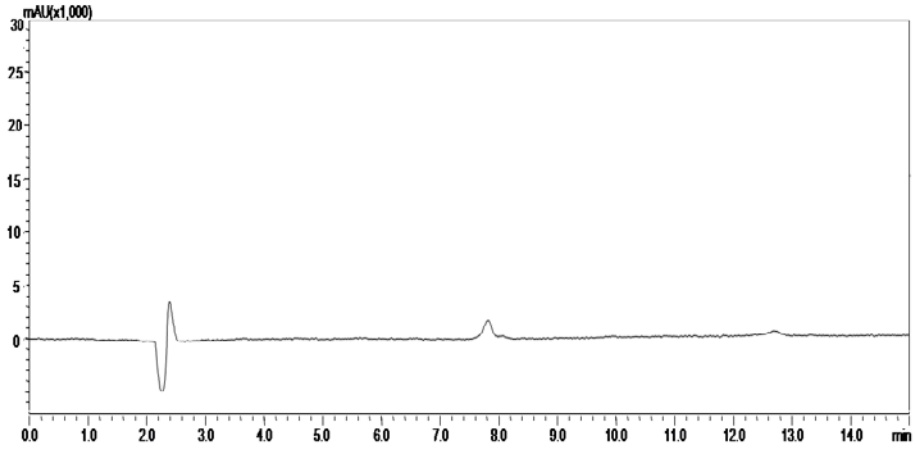

Figure 6: Chromatogram of Blank

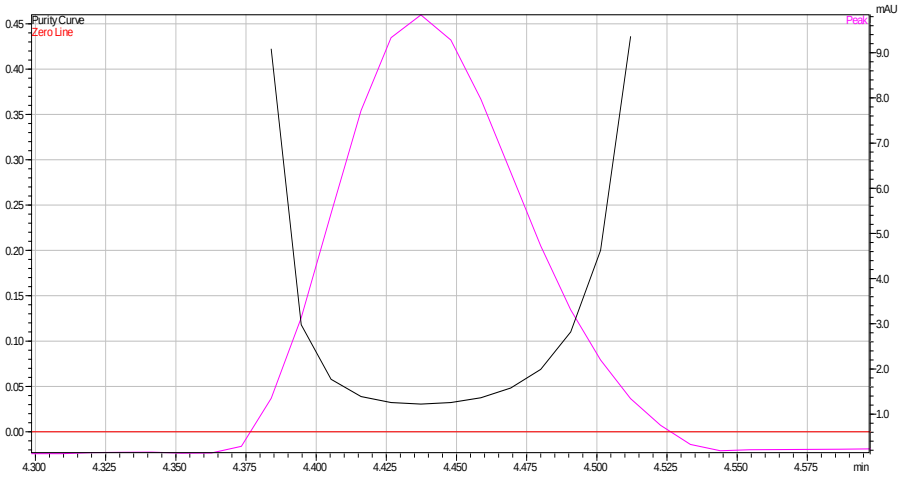

Figure 7: Peak purity of Piperacillin

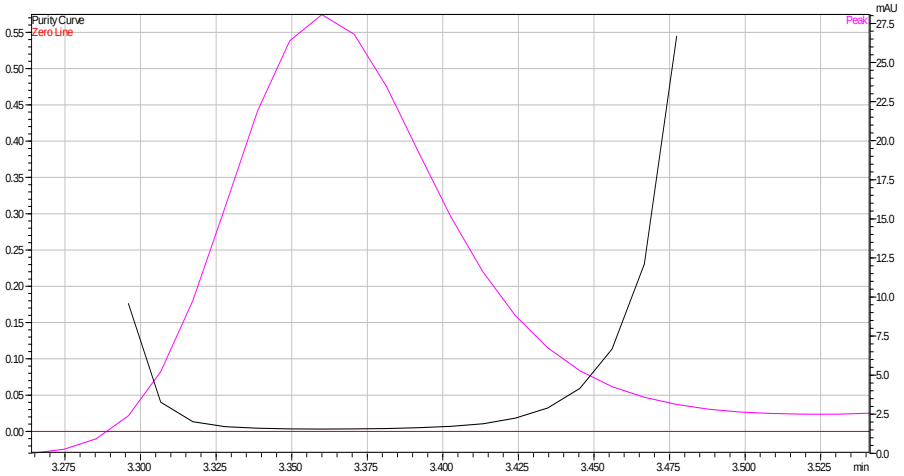

Figure 8: Peak purity for Tazobactam

Table 1: Optimized chromatographic conditions

CHROMATOGRAPHIC CONDITIONS	
Column	C_{18}Phenomenex (250 x 4.60mm)
Flow rate	1.0 ml/min
Run time	15min
Wavelength	225nm
Injection volume	10µl
Detector	PDA
Elution	Isocratic
Mobile phase	Acetonitrile, methanol and buffer pH 2.5 (50:30:20)
Column oven temperature	25 ± 5^0C

System suitability

System performance parameters of the developed HPLC method were determined by analyzing standard working solutions. Chromatographic parameters such as number of theoretical plates (N), resolution (Rs) and tailing factor were determined. The results are shown in Table 2.

Table 2: System suitable parameters of Piperacillin and Tazobactam Sodium

Parameters	Piperacillin	Tazobactam sodium
Linearity range	40-200 µg/ml	5-25 µg/ml
Regression equation	y=431.76x + 2370	y=1000.47x + 786.1
Slope	0.9996	0.9998
Intercept	431.76	1000.4
Correlation coefficient	2370	786.1
Retention time (min)	4.5±0.1	3.2±0.1
LOD (µg/ml)	0.625	0.0150
LOQ (µg/ml)	1.81	0.0455
Resolution	2.257	---
Theoretical plates	2014.112	2913.437
Tailing factor	1.652	1.711

Linearity

Under the chromatographic conditions described above, linear calibration curves for both piperacillin and tazobactam were obtained with five concentration level each. Peak area and concentration of each drug substance was subjected to regression analysis. The linearity range of Piperacillin was 40-200µg/ml and 5-25 µg/ml for tazobactam. The calibration graphs of piperacillin and tazobactam were shown in figure 9 & 10.

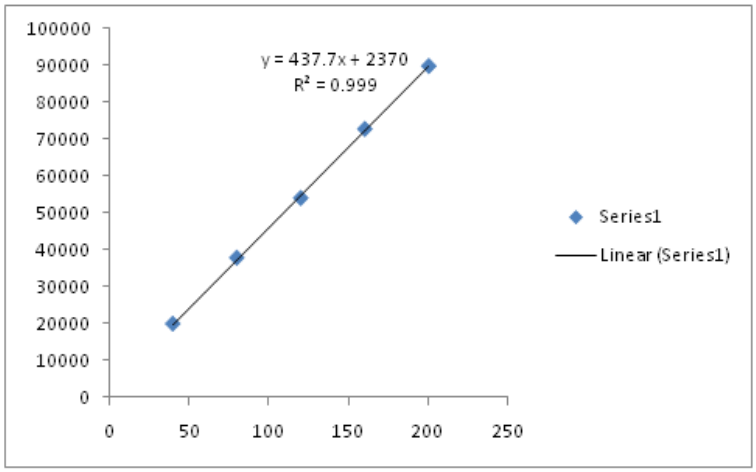

Figure 9: Calibration graph of Piperacillin in bulk drug (20-200µg/ml) at 225nm

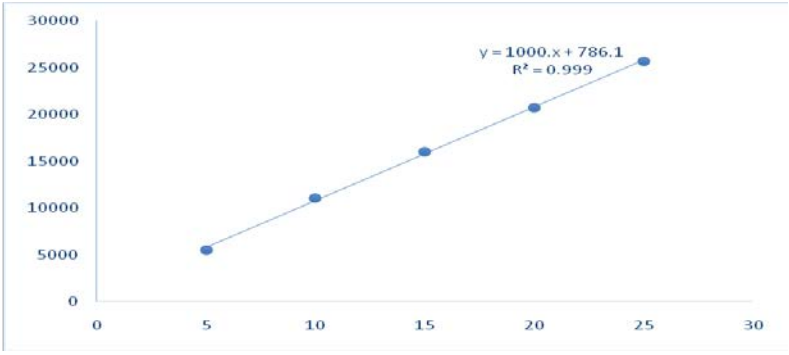

Figure 10: Calibration graph of Tazobactam sodium in bulk drug (5-25µg/ml) at 225nm

Accuracy

Accuracy should be across the specified range of the analytical procedure. The accuracy was then calculated as the percentage of analytes recovered by assay. Mean recoveries for piperacillin and tazobactam from the combination formulations are shown in Table 3 indicating good accuracy of the method.

Table 3: Recovery results for Piperacillin (PIP) and Tazobactam (TZB)

Level of % addition	Amount of Std drug taken (µg/mL)		Amount of drug added (µg/mL)		Total amount found (µg/mL)		% Recovery		%RSD	
	PIP	TZB	PIP	TZB	PIP	TZB	PIP	TZB	PIP	TZB
50	40	5	80	10	117.8	15.36	98.9	101.2	1.84	0.81
					124.8	14.97	102.4	99.9		
					119.2	14.91	99.6	99.7		
100	80	10	80	10	160.4	19.44	100.2	98.6	0.79	0.79
					156.8	20.16	98.7	100.4		
					159.7	20.32	99.9	100.8		
150	120	15	80	10	194.6	34.4	98.1	98.8	0.66	0.66
					201.4	35.05	100.5	100.1		
					199.1	34.6	99.7	99.2		

Precision

Precision of the method was determined with the product. An amount of the product powder equivalent to 50,100 and 150% of label claim was weighed accurately and assayed in six replicate determinations for each of three weighing amounts. The results for precision are shown in Table 4 & 5, indicating that acceptable precision was achieved for piperacillin and tazobactam as revealed by relative standard deviation data (RSD <2.0% in all of the levels of the two drugs).

Table 4: Intraday and Interday results for Piperacillin

		Intraday			Interday		
S.No	Amount of drug taken (µg/mL)	Peak Area	Mean	% RSD	Peak Area	Mean	% RSD
1	80	19870	19822	0.72	19774	19755	0.54
2		19887			19842		
3		19769			19679		
4		19964			19579		
5		19888			19870		
6		19559			19786		

Table 5: Intraday and Interday results for Tazobactam Sodium

		Intraday			Interday		
S.No	Amount of drug taken (µg/mL)	Peak Area	Mean	% RSD	Peak Area	Mean	% RSD
1	10	11067	11066	0.25	11053	11069	0.17
2		11078			11076		
3		11096			11097		
4		11089			11086		
5		11025			11055		
6		11041			11052		

Table 6: Analysis of marketed formulations

Brand name	Label claim (mg)		Amount found (mg)		% Assay	
	Piperacillin	Tazobactam	Piperacillin	Tazobactam	Piperacillin	Tazobactam
Tazar	4000	500	3898.40	490.92	97.64	97.13
Durataz	4000	500	3988.00	501.00	99.97	100.2
Tazact	2000	250	2021.00	252.47	101.05	100.99

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD was calculated to be 0.625µg/ml for piperacillin and 0.0150µg/ml for tazobactam. The LOQ of piperacillin and tazobactam were found to be 1.81µg/ml and 0.0455µg/ml respectively.

Robustness

The robustness was determined by carrying out the assay during which the mobile phase ratio, pH of the mobile phase, column oven temperature and flow rate were altered slightly. The results were tabulated in Table 7.

Table 7: Results for Robustness of Piperacillin and Tazobactam

	Piperacillin		Tazobactam	
Condition	**Tailing**	**%RSD**	**Tailing**	**%RSD**
Optimized method	1.652	---	1.711	---
Wavelength				
Decreased 2nm	1.650	0.17	1.710	0.04
Increased 2nm	1.646	0.24	1.717	0.23
Buffer pH				
Decreased (-0.5 units)	1.634	0.77	1.752	1.67
Increased (+0.5 units)	1.637	0.64	1.741	1.22
Flow rate				
Decreased (-0.1 ml/min)	1.689	1.56	1.721	0.61
Increased (+0.1 ml/min)	1.663	1.72	1.732	0.86
Column temperature				
Decreased (-5^0 C)	1.666	0.59	1.759	1.69
Increased (+5^0 C)	1.638	0.77	1.776	1.45
Mobile phase ratio (MET: ACN: 1%Orthophosphoric acid)				
Decreased (2%)	1.642	0.42	1.706	0.20
Increased (2%)	1.671	0.78	1.763	1.73

CONCLUSION

The developed RP-UFLC-DAD method for the estimation of piperacillin and tazobactam sodium offers simplicity, selectivity, precision and accuracy in bulk and injection dosage forms. This method was validated as per ICH guidelines. Thus it can be used for routine quality control studies for the assay of piperacillin and tazobactam sodium.

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