

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CEFTAZIDIME AND AVIBACTAM IN PHARMACEUTICAL DOSAGE FORM BY UV VISIBLE SPECTROPHOTOMETRIC METHOD**Mrs. N. Sandhyarani*, Dilshad Begum, B. Kavya, Sumaiya Begum, K. Anusha, D. Mayuri Ramulu**

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DOI: <https://doi.org/10.5281/zenodo.20963887>**How to cite this Article:** Mrs. N. Sandhyarani*, Dilshad Begum, B. Kavya, Sumaiya Begum, K. Anusha, D. Mayuri Ramulu. (2026). Method Development and Validation For Simultaneous Estimation of Ceftazidime and Avibactam In Pharmaceutical Dosage Form By Uv Visible Spectrophotometric Method. World Journal of Pharmaceutical and Medical Research, 12(7), 322-328.

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Article Received on 27/05/2026

Article Revised on 16/06/2026

Article Published on 01/07/2026

ABSTRACT

Ceftazidime and Avibactam are combination antibiotic used to treatment of serious infections caused by susceptible gram – negative bacteria, including many multidrug resistant strains. Avibactam protects ceftazidime from bacterial β - lactamase enzyme thereby extending its activity. The present study aimed to develop and validate a simple, rapid, accurate, and economical UV spectrophotometric method for the estimation of ceftazidime and Avibactam in bulk and pharmaceutical dosage form. Ceftazidime and avibactam were dissolved in phosphate buffer (PH 7.0) and suitably to obtain the required concentrations and maximum absorbance were shown at 257nm and 226nm respectively. The developed method obeyed Beer–Lambert’s law over a concentration range of 2–20 $\mu\text{g/mL}$. Method validation was performed according to pharmaceutical regulatory guidelines, evaluating parameters such as linearity, accuracy, precision, sensitivity, limit of detection (LOD), and limit of quantification (LOQ). The calibration curve showed good linearity with a regression coefficient (R^2) of 0.987. Accuracy was assessed through recovery studies, with percentage recoveries ranging from 98.7% to 101%. Precision studies demonstrated satisfactory reproducibility, with %RSD values less than 2%. The method exhibited good sensitivity, with the limit detection was 6.2215 $\mu\text{g/ml}$ and 6.23771 $\mu\text{g/ml}$ respectively. The limit of qualification was found to be 18.853 $\mu\text{g/ml}$ and 18.902 $\mu\text{g/ml}$. respectively. The results demonstrated satisfactory recovery, low relative standard deviation (%RSD), and good reproducibility, indicating the reliability of the method. No interference from formulation excipients was observed, confirming the specificity of method. Owing to its simplicity, accuracy, precision, cost effective and reliability, the proposed UV spectrophotometric method is suitable for quality control analysis of ceftazidime and avibactam in bulk drug and pharmaceutical dosage form.

KEYWORDS: Ceftazidime, Avibactam, UV spectrophotometry, simultaneous equation method, Method validation, ICH guidelines.**1. INTRODUCTION**

Simultaneous estimation plays an important role in pharmaceutical industry because it is very feasible and time saving method. Spectrophotometric methods and Chromatographic methods provide high degree of assurance that these techniques fit for the simultaneous estimation in the pharmaceutical dosage form.^[1]

Ceftazidime chemically, ((6R,7R)-7-[[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(2-carboxypropan-2-yloxyimino)acetyl] amino]-8-oxo-3-(pyridin-1-ium-1-ylmethyl)-5-

thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate) works by attacking the bacterial cell wall, which is essential for the bacteria to survive. It behaves like a "molecular wrench" that jams the enzymes responsible for building and cross-linking the cell wall's protective layer. Because the bacteria can no longer strengthen their outer shell as they grow or divide, they become structurally weak. Eventually, the internal pressure of the cell causes it to swell and burst (lysis), effectively killing the bacteria. This specific action is highly effective against tough Gram-negative bugs like Pseudomonas because

ceftazidime is designed to resist the "shields" (enzymes) those bacteria use to try and destroy the antibiotic. Severe pneumonia, Complicated and uncomplicated urinary tract infections, Skin and soft tissue infections, Complicated intra-abdominal infections Empiric treatment for neutropenic fever.^[2,3]

Avibactam chemically, ((2S,5R)-2-carbamoyl-7-oxo-1,6-diazabicyclooctan-6-yl] hydrogen sulphate.) works by acting as a decoy for bacterial enzymes called beta-lactamases, which normally chew up and destroy antibiotics like penicillin or cephalosporins. Unlike older inhibitors that get destroyed during this process, Avibactam uses a unique reversible binding mechanism: it latches onto the enzyme, pinches it shut to stop its activity, and can even let go and "reset" to disable another enzyme later. By keeping these enzymes busy or broken, it clears a path for partner antibiotics (like Ceftazidime) to reach and kill the bacteria effectively.^[4,5]

2. MATERIALS AND METHODOLOGIES

Chemicals and reagents

Ceftazidime and Avibactam were purchased from Patel's Scientific Traders, Hyderabad. Tablet dosage form of brand Ceftazil-AV was purchased from local pharmacy. Phosphate buffer tablets and distilled water were supplied by SD Fine chemicals, Mumbai.

Instruments

For the experiment, a Lab India Analytical UV-Visible spectrophotometer(double-beam)- with a pair quartz cell having a 1cm length was used. Weighing was carried out using CONTECH electronic balance.

Preparation of Standard solution of Ceftazidime and Avibactam

Both Ceftazidime(10mg) and Avibactam (10mg) were weighed carefully and transferred to separate 10ml volumetric flask and diluted with Phosphate buffer

solution of Ph7 to get the concentration of 1000µg/ml. This concentration is diluted in a separate volumetric flask to attain 100µg/ml (Stock - A). The stock solution is further diluted with solvent to attain desired concentrations.

Preparation of sample solutions

10mg equivalent injection powder of Ceftazil-AV was transferred to 10ml volumetric flask and dissolved it in Phosphate buffer solution and made up the solution to 10 ml with solvent. From this further dilutions were made to get the desired concentrations for both the drugs.

METHODOLOGY

Simultaneous Equation Method

The simultaneous equation was applied to evaluate Ceftazidime and Avibactam Concentrations in the injection dosage form. The UV Spectra were recorded between 200-400 nm. Maximum absorbances were seen at 257nm and 226nm respectively. The below mentioned formula were used to determine the exact amount of medication in dosage form.

$$C_x = (A_2 a_{y1} - A_1 a_{y2}) / (a_{x2} a_{y1} - a_{x1} a_{y2})$$

$$C_y = (A_1 a_{x2} - A_2 a_{x1}) / (a_{x2} a_{y1} - a_{x1} a_{y2})$$

C_x and C_y represent the amounts of Ceftazidime and Avibactam in the sample solutions respectively. The absorbance of sample solution at 257nm is A_1 and at 226nm is A_2 . Absorptivity of Ceftazidime at 257 nm and 226 nm were denoted by a_{x1} and a_{x2} . Absorptivity of Avibactam at 257nm and 226 nm were denoted by a_{y1} and a_{y2} .

3. RESULTS AND DISCUSSIONS

Selection of wavelength

The absorbance of the solutions containing Ceftazidime 20µg/ml and Avibactam 20µg/ml were scanned separately in the UV range 200-400nm using Phosphate buffer as blank.

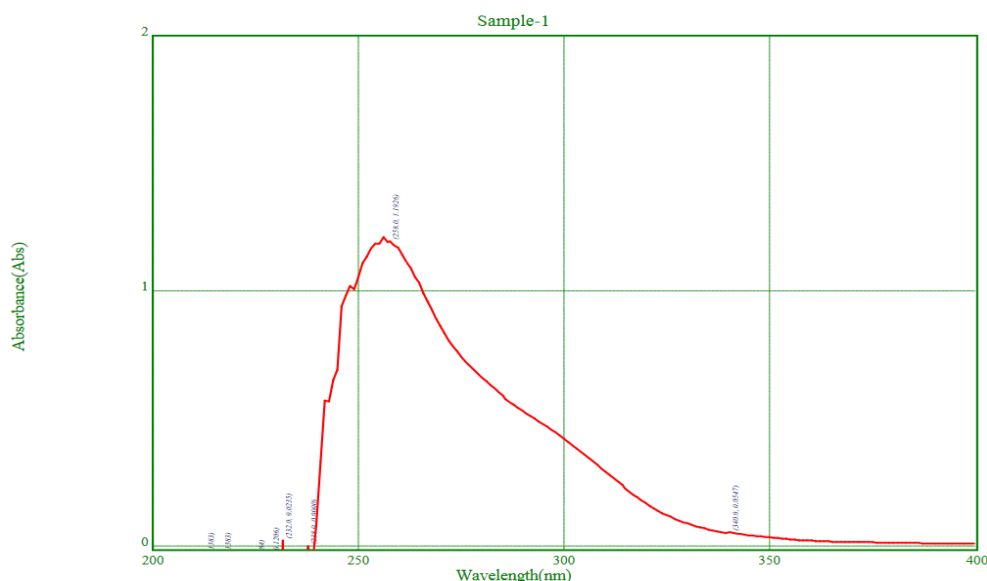


Figure no. 1: Figure showing λ_{max} of Ceftazidime.

For the Ceftazidime maximum wavelength was found at 257 nm, and maximum absorbance was found to be 1.1926

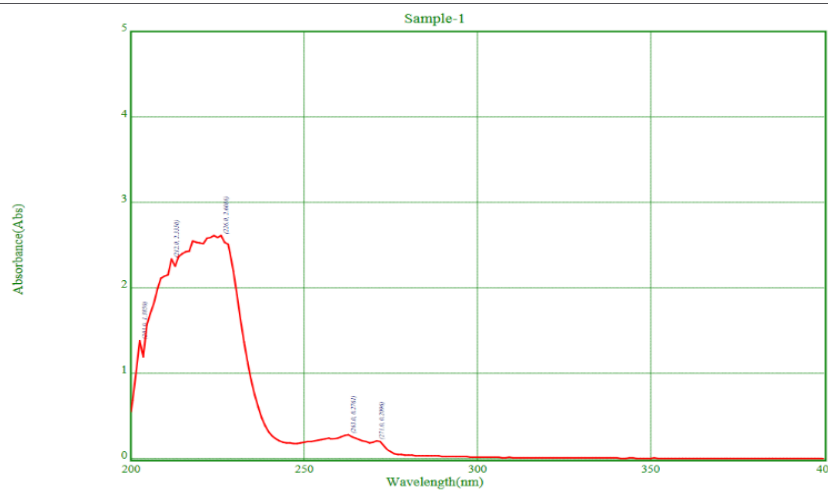


Figure no. 2: Figure showing λ_{max} of Avibactam.

For the Avibactam, maximum wavelength was found at 226 nm, and maximum absorbance was found to be 2.608

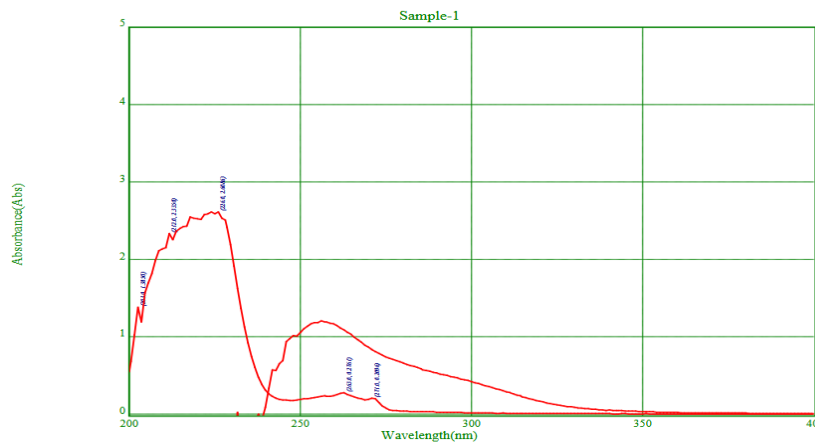


Figure no. 3: Figure showing isobestic point of Ceftazidime and Avibactam.

The isobestic point was observed at 240nm by using overlapping method.

ASSAY

Table No. 1: Observation results for Assay.

S. No.	Drug	Conc.	Absorbance		Absorptivity	
			226nm	257nm	226nm	257nm
1.	Ceftazidime	10 μ g/ml	0.05159	0.122051	0.5159 (ax ₁)	0.122051(ax ₂)
2.	Avibactam	10 μ g/ml	0.05565	0.044111	0.05565(ay ₁)	0.044111(ay ₂)
3.	Injection formulation	Unknown (Cx)	0.128 (A1)	0.735 (A2)		
	Injection formulation	Unknown (Cy)	0.128 (A1)	0.735 (A2)		

$$C_x = (A_2 ay_1 - A_1 ay_2) / (ax_2 ay_1 - ax_1 ay_2)$$

$$= 7.86\mu\text{g/ml}$$

$$C_y = (A_1 ax_2 - A_2 ax_1) / (ax_2 ay_1 - ax_1 ay_2)$$

$$= 2.12\mu\text{g/ml}$$

Assay of Ceftazidime and Avibactam

= (Concentration x average weight in mg) / (Weight of powder equivalent to 10mg x label claim) X 100

The percentage purity of Ceftazidime and Avibactam in injection dosage form were found to be 98.5% and 106% respectively by using simultaneous equation method.

VALIDATION PARAMETERS SPECIFICITY

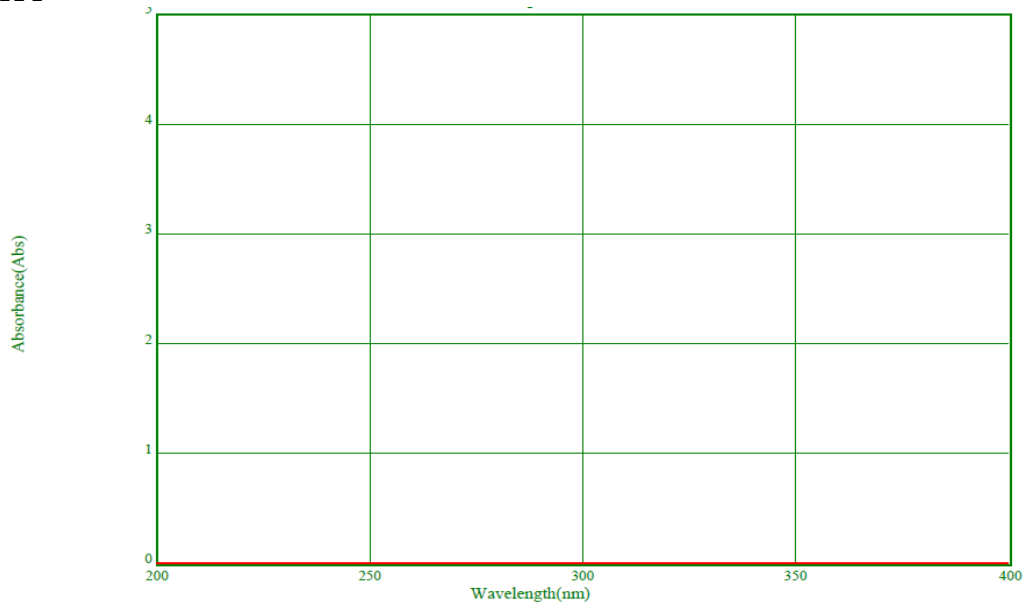


Figure no. 4: Spectrum showing blank.

Observation: From the spectrum we can conclude that Ceftazidime and Avibactam. excipients or solvents are not interfering the spectrum of

LINEARITY

Table No. 2: Observation values for Linearity.

S.No	Conc. [$\mu\text{g/ml}$]	Absorbance of Cef at 257nm	Absorbance of Avi at 226nm
1	2	0.175	0.121
2	4	0.286	0.272
3	6	0.398	0.316
4	8	0.499	0.406
5	10	0.595	0.515
6	12	0.693	0.625
7	14	0.752	0.789
8	16	0.863	0.846
9	18	0.998	0.927
10	20	1.120	1.218

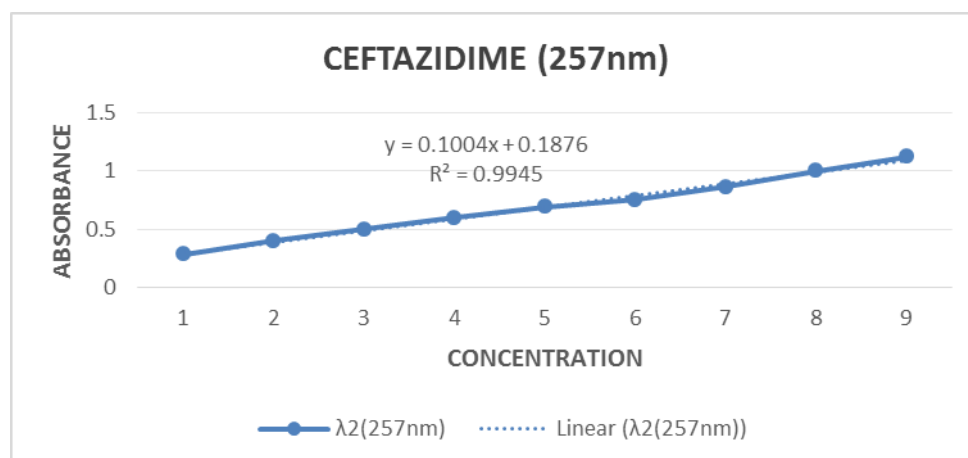


Fig. no. 5: Calibration Curve of Ceftazidime.

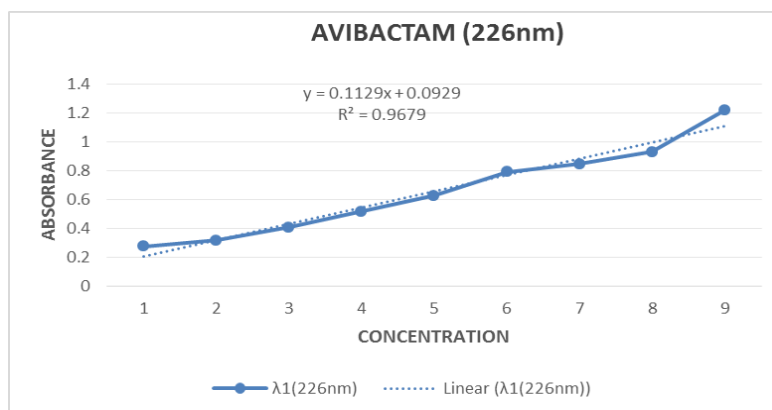


Fig. no. 6: Calibration Curve of Avibactam.

ACCURACY

Table No. 3: Observation values for Ceftazidime.

Level of spiking	conc. (µg/m)	STANDARD		SPIKED		TEST		% Recovery	
		λ1(226)	λ2(257)	λ1(226)	λ2(257)	λ1(226)	λ2(257)	λ1(226)	λ2(257)
50%	1	0.324	0.356	0.921	0.75	0.128	0.565	100%	100%
	2	0.318	0.348	0.932	0.752	0.124	0.567	101.8%	104.88%
	3	0.318	0.353	0.928	0.748	0.122	0.564	105%	103.11%
100%	1	0.589	0.595	1.16	0.981	0.129	0.561	99.8%	100.6%
	2	0.548	0.586	1.151	0.983	0.127	0.567	100.1%	99.6%
	3	0.583	0.566	1.157	0.985	0.123	0.566	100.8%	99.8%
150%	1	0.776	0.776	1.348	1.254	0.126	0.57	100.2%	100.2%
	2	0.762	0.762	1.35	1.258	0.122	0.574	100.78%	101.8%
	3	0.773	0.773	1.344	1.25	0.129	0.569	99.87%	100.25%

Table No. 4: Table showing results of accuracy of Avibactam.

Level of spiking	conc. (µg/m)	STANDARD		SPIKED		TEST		% Recovery	
		λ1(226)	λ2(257)	λ1(226)	λ2(257)	λ1(226)	λ2(257)	λ1(226)	λ2(257)
50%	1	0.298	0.185	0.426	0.75	0.129	0.564	99.6%	100.5%
	2	0.287	0.187	0.415	0.752	0.128	0.567	100%	98.9%
	3	0.994	0.183	0.422	0.748	0.124	0.562	101.3%	101.6%
100%	1	0.515	0.416	0.643	0.981	0.127	0.569	100.19%	99.03%
	2	0.514	0.418	0.642	0.983	0.126	0.561	100.3%	100.95%
	3	0.517	0.42	0.645	0.985	0.122	0.567	101.16%	99.5%
150%	1	0.801	0.689	0.929	1.254	0.12	0.563	100.9%	100.2%
	2	0.811	0.693	0.939	1.258	0.123	0.566	100.6%	99.8%
	3	0.805	0.685	0.933	1.25	0.125	0.565	100.3%	100%

The results obtained for recovery at 50%, 100%. 150% are within the limits. Hence method is accurate.

PRECISION

Table No. 5: Observation values for Repeatability of Ceftizidime and Avibactam.

S _p No	Conc. (µg/ml)	Absorbance of cef at 257nm	Absorbance of Avi at 226nm
1	10	0.0651	0.0523
2	10	0.0654	0.0526
3	10	0.0648	0.0521
4	10	0.0653	0.0527
5	10	0.0650	0.0525
	Average	0.06512	0.05244
	SD	0.00024	0.00024
	%RSD	0.37%	0.46%

INTERMEDIATE PRECISION**Table No. 6: Observation values for Intermediate Precision.**

Concentration	Ceftazidime		Avibactam	
	Day-1	Day-2	Day-1	Day-2
10 µg/ml	0.0651	0.0652	0.0523	0.0522
10 µg/ml	0.0654	0.0650	0.0526	0.0525
10 µg/ml	0.0648	0.0649	0.0521	0.0520
10 µg/ml	0.0653	0.0655	0.0527	0.0524
10 µg/ml	0.0650	0.0651	0.0525	0.0523
Average	0.06512	0.06514	0.05244	0.05228
SD	0.00024	0.000230	0.00024	0.000192
%RSD	0.37%	0.35%	0.46%	0.37%

The %RSD obtained is within the limit. Hence the method is precise.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION(LOQ)**Table No. 7: Observation values for LOD & LOQ.**

Parameter	Ceftazidime	Avibactam
Standard Deviation	0.002639444	0.002160247
Slope	0.002639444	0.002160247
LOD	0.002639444	0.002160247
LOQ	18.85317419	18.85317419

ROBUSTNESS**Table No. 8: Observation values for Robustness.**

Conc.	CEFTAZIDIME		AVIBACTAM	
	Wavelength 258 nm	Wavelength 260 nm	Wavelength 224 nm	Wavelength 228 nm
10 (µ/ml)	0.0555	0.0552	0.0424	0.0421
10 (µ/ml)	0.0553	0.0556	0.0428	0.0419
10 (µ/ml)	0.0551	0.055	0.0425	0.042
10 (µ/ml)	0.0552	0.0557	0.0426	0.0429
10 (µ/ml)	0.0557	0.0555	0.0421	0.0425
10 (µ/ml)	0.0554	0.0551	0.0422	0.0427
AVERAGE	0.055366667	0.05535	0.042433333	0.04235
SD	0.000216025	0.000288097	0.000258199	0.000408656
% RSD	0.390171023	0.520500824	0.60848128	0.964950023

4. SUMMARY AND CONCLUSION**SUMMARY**

A UV spectrophotometer method has been developed and validated for the simultaneous estimation of ceftazidime and combination drug (ceftazidime and avibactam) in its pure and tablet dosage form.

The process was done by using simultaneous equation method with the detection wavelength set at 257nm, 226nm for ceftazidime and combination drug. The method was linear with correlation coefficient 0.987 in the concentration range of 2µg -20µg in this method. The limit detection were 6.2215µg/ml and 6.23771µg/ml respectively. The limit of qualification were found to be 18.853µg/ml and 18.902µg/ml respectively. The repeatability of inter day 1 and day 2 precision were satisfactory and the relative standard deviation did not exceed 2%. The accuracy of the method from the recovery studies is within the limits for both the drugs and stand also at isosbestic point. The two methods are robusted. The methods met the ICH regulatory requirements.

CONCLUSION

A simple, accurate, precise, reproducible, robust and economical UV spectrophotometer methods for simultaneous methods for simultaneous estimation of ceftazidime and combination of drugs in the pure and pharmaceutical dosage form have been developed. The method is simultaneous equation method. The method was developed by using phosphate buffer PH-7. The developed method was validated for parameters viz accuracy, precision, linearity, robustness, limit of detection an limit of qualification as per ICH guidelines. All the parameters were found to be within the acceptance limits. The results indicated that the proposed method for simultaneous estimation of ceftazidime and combination of drugs are very accurate and cost effective and can be employed in routine sample analysis in its pure and pharmaceutical dosage form.

ACKNOWLEDGMENT

Our sincere thanks to our SSRCP management for providing us power and strength to overcome all the

hurdles and hindrances that come in the way of doing project.

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