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Research Article

UV-visible derivative spectroscopic method for simultaneous estimation of cefoperazone and tazobactam in injection dosage form

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ABSTRACT

A simple, accurate, rapid, economical UV spectrophotometric method namely Absorption ratio and RP-HPLC method have been developed and validated for estimation of Cefoperazone and Tazobactam in injection dosage form and can be used in routine analysis. Cefoperazone is 3rd generation of cephalosporin containg drug used in Septicemia, Biliary tract infection and urinary tract infection and Tazobactam is a Antibacterial agent used along with cefoperazone in emergency. It is manufactured by Biocon Pharmaceuticals (New Delhi) under the brand name Cegava-Tz. The ZCPs for CEFO and TAZO were found to be 320 nm and 232.8 nm respectively. Results of the validation of the above method indicate that the method was linear in the range of 25-75 µg/ml for CEFO and 3.125- 9.75 µg/ml for TAZO. The % recoveries for CEFO and TAZO obtained in the accuracy study were 97.43 – 98.82 % and 95.46 - 100.2% respectively. The high precision of proposed method is confirmed by % RSD below 2.0 for repeatability.
The % assay results of 97.84 % for CEFO and 100.25 % for TAZO indicate the proposed derivative spectroscopic method was simple, accurate, pricise and successfully applied for determination of CEFO and TAZO from Cegava-Tz injection dosage form forroutine analysis.

Keywords: UV Spectrophotometric; Cefoperazone, Tazobactam; Validation; Derivative Method

INTRODUCTION

Cefoperazone

Cefoperazone (CEFO), is an anti-Bacterial, Beta Lactam Antibiotic, is commonly used for Septicaemia, Lower respiratory tract, upper and lower urinary tract infections, Pneumonia, Meningitis, Biliary tract infection. Structure of Cefoperazone shown in figure: 1 [1-5].
It is official in Official in Indian Pharmacopoiea, British pharmacopoeia and United state pharmacopoeia/National formulary [6,7,8].

Mechanism of Action

Cefoperazone inhibit bacterial cell wall formation. Peptidoglycan is a component of the cell wall and provide mechanical stability by virtue of its highly cross linked
Lattice work structure.
Cefoperazone inhibit enzyme transpeptidase that brings about cross-linking between 5th glycine of the existing Peptidoglycan in the cell wall and 4th amino acid of the newly formed Peptidoglycan. It inhibit the peptidoglycan synthesis. [1,2]

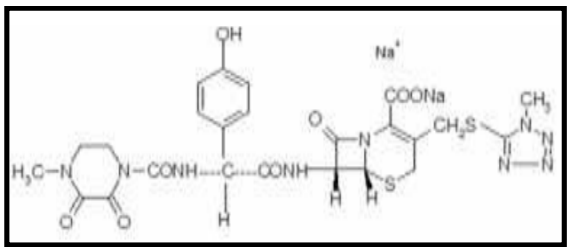

Figure No.: 1 "Structure of Cefoperazone"

Tazobactam

Tazobactam (TEZO) is an Anti microbial, β lactamase inhibitors. It is an effective anti-microbial agent, It is used in Abdominal infection, Respiratory tract infection, septicaemia, skin infection, urinary tract infection. Structure of Tazobactam shown in figure: 2 [1,2,3,4,9]. It is official in United state pharmacopoeia / National formulary [8].

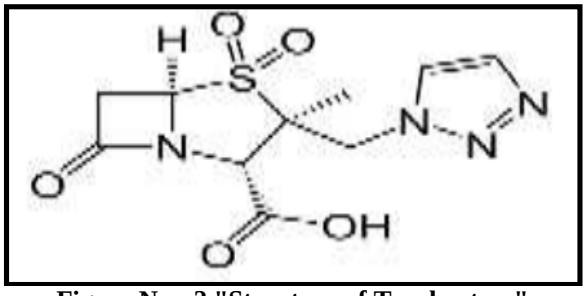

Figure No. :2 "Structure of Tazobactam"

Mechanism of Action

They bind with beta lactamase enzyme and inactive them. Hence they prevent the destruction at beta lactam antibiotics [1,2].

Introduction Dosage Form

Cegava-Tz Injection is used as antibiotic formulation. Cegava-Tz Injection contains Cefoperazone and Tazobactam as active ingredients. The emergence and subsequent widespread dissemination of bacterial resistance to a variety of beta-lactam antibiotics by the elaboration of ESBL (Extended Spectrum Beta Lactamase) poses a serious threat to the effective use of beta lactam antibiotics and cephalosporins. Cefoperazone combination with the beta lactamase inhibitor tazobactam would be a strong basis for rational therapeutics when dealing with ESBL producing pathogen mediated infections. The beta lactamase inhibitor tazobactam in improving the spectrum of activity and efficacy of cefoperazone. The Combination is approved by CDSCO in 4/11/2009 [10].

Available Marketed Formulation
Brand Name: Cegava-Tz
Manufacturer: Biocon Pharma Limited
Ratio of Drug: Cefoperazone : Tazobactam (1000 mg : 125 mg)

The review of literature revealed that couple of analytical methods including UV-Spectrophotometry and HPLC have been reported for Cefoperazone and Tazobactam individually or with other combinations.
But there is no derivative UV method validation method was reported for this combination of drugs. So, there is cospicuous regerance of interest to develop simple and cost effective Derivative UV-Visible spectroscopy method.

MATERIALS AND METHODS

Selection of Solvent
Cefoperazone and Tazobactam are soluble in methanol therefore methanol is selected as solvent.

Preparation of standard stock solution of Cefoperazone
Accurately weighed Cefoperazone (50 mg) was accurately weighed and transferred to a 100 ml volumetric flask and dissolved and diluted up to the mark with methanol to produced 500 µg/ml standard stock solution of Cefoperazone. 1.0 ml aliquots of the solution was taken and transferred to 10 ml volumetric flask from stock and make up volume with methanol to produced 50 µg/ml solution of Cefoperazone.

Preparation of standard stock solution of Tazobactam
Accurately weighed Tazobactam (6.25 mg) was accurately weighed and transferred to a 100 ml volumetric flask and dissolved and diluted up to the mark with methanol to produced 62.5 µg/ml standard stock solution of Tazobactam. 1.0 ml aliquots of the solution was taken and transferred to 10 ml volumetric flask from stock and make up volume with methanol to produced 6.25 µg/ml solution of Tazobactam.

Selection of analytical wavelength
The standard solutions of Cefoperazone (50 µg/ml) and Tazobactam (6.25µg/ml) were scanned separately in the UV range of 200-400 nm. The zero order spectra was obtained, then processed to obtain First Order Derivative spectrum. It appeared that Cefoperazone having Zero Crossing Point (ZCP) at 320 nm while Tazobactam having Zero Crossing Point (ZCP) at 232.8 nm**.**

Calibration curve for Cefoperazone and Tazobactam
Accurate volume (0.5, 0.75, 1, 1.25, 1.5 ml) from standard stock solution of Cefoperazone and Tazobactam was transferred to volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the Methanol to obtain concentration of 25, 37.5, 50, 62.5 and 75 µg/ml of Cefoperazone and 3.125, 4.687, 6.25, 7.812, 9.375 µg/ml of Tazobactam. The zero order spectra was obtained, then processed to obtain First Order Derivative spectrum. Absorbance of each solution was measured at 320.0 nm for Cefoperazone (Zero crossing Point of Cefoperazone and 232.8 nm for the Tazobactam (Zero crossing Point of Tazobactam) using first order derivative spectrophotometry. The graph of absorbance verses concentration was plotted.

Sample preparation for determination of Cefoperazone and Tazobactam in combined dosage form

- Each bottle containing Cefoperazone and Tazobactam in ratio of 1000:125 mg respectively was weighed..Take Powder equivalent to 50 mg of Cefoperazone and 6.25 mg of Tazobactam was weighed and transferred in a 100 ml volumetric flask and methanol was added.
- This solution was sonicated for 15 minutes and final volume was made to the mark with methanol. The solution was filtered through Whatman filter paper No. 41.
- The filtrate 1 ml was transferred in a 10 ml volumetric flask and diluted to the mark with methanol to obtain Cefoperazone (50 µg/ml) and Tazobactam (6.25 µg/ml). Concentration was calculated by regression equation method and % Assay was calculated.

Validation of spectrophotometric method [11, 12]

Linearity and range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity. Selected linearity range for Cefoperazone was 25-75 µg/ml and for Tazobactam it was 3.125-9.375µg/ml.

Precision

Repeatability (n = 6)

For the repeatability study, the standard solutions of Cefoperazone and Tazobactam were utilised. From the standard solution of both drugs respectively, aliquot of 1 ml was transferred to a separate 10 ml volumetric flask and diluted up to mark with methanol such that it gives the concentration of 50 µg/ml of Cefoperazone and 6.25 µg/ml of Tazobactam. The procedure was repeated six times and % RSD was calculated.

Intraday Precision (n = 3)

From the standard stock solutions of Cefoperazone and Tazobactam, aliquots of 0.75, 1, 1.25, ml were transferred to separate 10 ml volumetric flasks and diluted up to the mark with methanol to give the concentration of 37.5, 50, 62.5 µg/ml for Cefoperazone and 4.687, 6.25, 7.812 µg/ml for Tazobactam. The solutions were analyzed three times on the same day and % RSD was calculated.

Interday Precision (n = 3)

From the standard stock solutions of Cefoperazone and Tazobactam, aliquots of 0.75, 1, 1.25, ml were transferred to separate 10 ml volumetric flasks and diluted up to the mark with methanol to give the concentration of 37.5, 50, 62.5 µg/ml µg/ml for Cefoperazone and 4.687, 6.25, 7.812 µg/ml for Tazobactam. The solutions were analyzed on three different days and % RSD was calculated.

LOD and LOQ

The LOD and LOQ of developed method were studied as per ICH guidelines. Several approaches for determining the LOD & LOQ are possible, depending on the procedure i.e, a non-instrumental or instrumental. Among them here employed method was,

LOD= $3.3\sigma/S$ and

LOQ= $10\sigma/S$

Where, σ = the standard deviation of response

S = the slope of calibration curve.

Accuracy

Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by addition of standard drug solution at the level of 80%, 100% and 120% to the preanalysed sample. Results of the recovery study were found to be within the acceptance criteria 100±10 %, indicating a good degree of sensitivity of the method towards detection of analyte in sample. In this method the known concentration of standard drug was added to the assay sample. The amount present was calculated and the assay amount was subtracted from it, which gives the amount recovered.

RESULTS AND DISCUSSION

Both drug shows good solubility in methanol so, it was used for method development. For estimation of Cefoperazone and Tazobactam, derivative spectroscopic method was employed.

Selection of Wavelength for Determination

Linear correlation was obtained between First derivative (D1) absorbance versus Concentrations of Cefoperazone and Tazobactam in the ranges of 25-75 µg/ml and 3.125-9.375 µg/ml. Regression parameters are mentioned in Table 1 & 2 and the calibration curves of Cefoperazone and Tazobactam are shown in Figure 6 & 7 respectively.

Table No. 1: "Lambert-Beer's curve Data for CEFO"

Conc. (µg/ml)	Mean Response ±RSD	% RSD
25	0.0090 ± 0.00089442	0.989
37.5	0.0100±0.00089442	0.893
50	0.01208±0.00010954	0.956
62.5	0.01402±0.00044721	0.318
75	0.01604±0.00089442	0.557

Table No. 2: "Lambert-Beer's curve data for TAZO"

Conc. (µg/ml)	Mean Response ± RSD	% RSD
3.125	0.00504± 0.000547723	1.086
4.687	0.00698± 0.000447214	0.640
6.25	0.1004±0.000547723	0.545
7.812	0.1204 ±0.0005572	0.454
9.375	0.1406 ±0.000547723	0.389

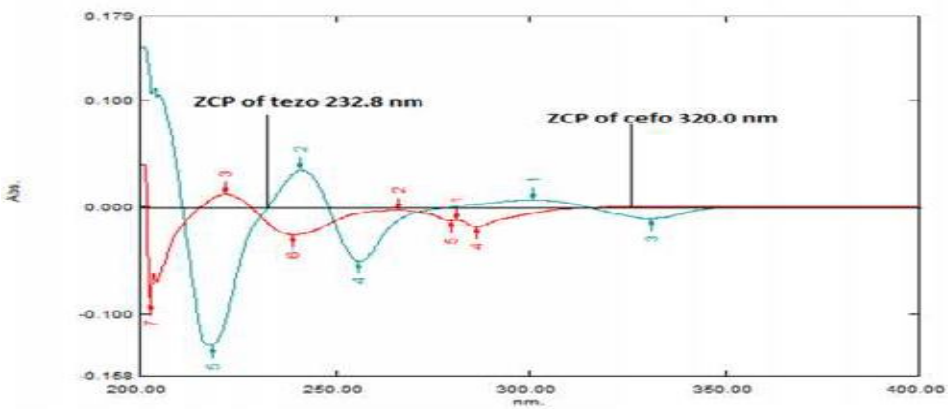

Figure No. 3: "Overlain First Derivative (D1) Absorption Spectra of Standard Cefoperazone (50 µg/ml) and Tazobactam (6.25 µg/ml)"

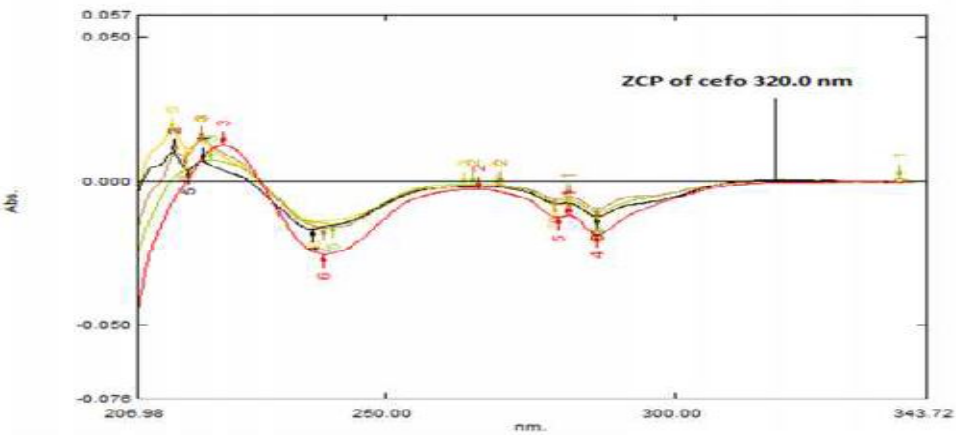

Figure No. 4: "Overlain spectra of Cefoperazone"

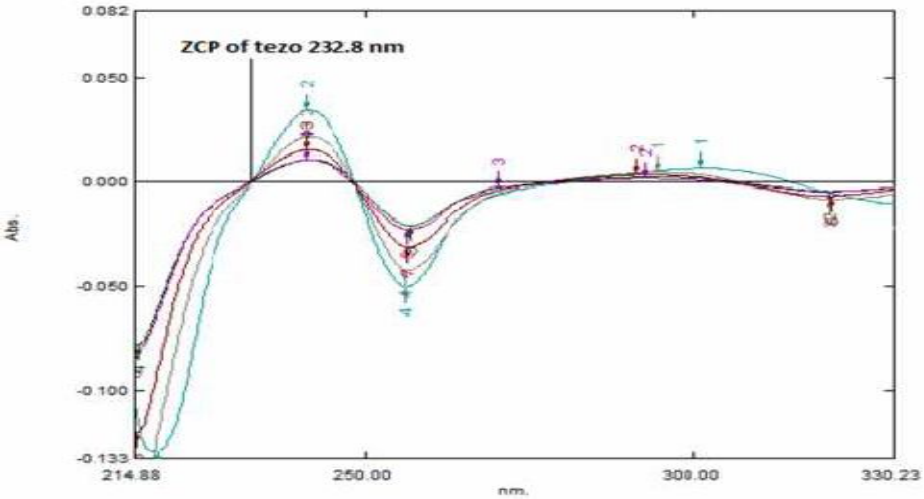

Figure No. 5: "Overlain spectra of Tazobactam"

Linearity and Range (n = 5)

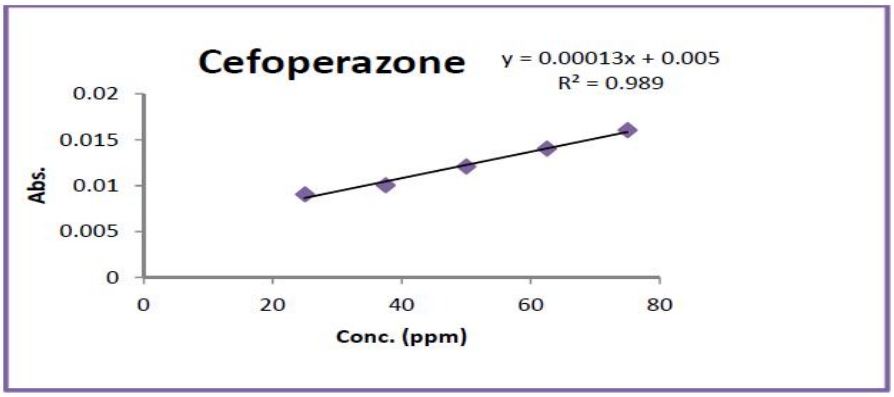

Figure No. 6 : "Calibration curve of Cefoperazone"

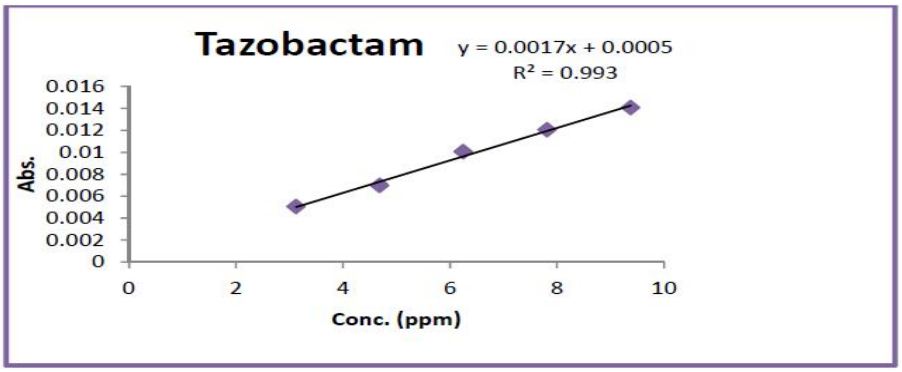

Figure No. 7 : "Calibration curve of Tazobactam"

Precision

Repeatability (n = 6)

The repeatability data for CEFO and TAZO are shown in Table 3. The % RSD was found to be 0.938 % for CEFO and 0.645 % for TAZO.

Table No. 3: "Repeatability Study"

Concentration (µg/ml)	Cefoperazone		Tazobactam	
	Mean ± SD (n = 6)	% RSD	Mean ± SD (n = 6)	% RSD
50 CEFO 6.25 TAZO	0.01216 ± 0.000109 45	0.938	0.01004 ± 0.000547 21	0.645

Intraday precision and Interday precision (n = 3)

The data for Intraday and Interday precision for CEFO is shown in Table 4. The % RSD for Intraday and Interday precision was found to be 0.318 – 0.9068 and 0.63 – 1.40 for CEFO. The data for Intraday and Interday precision for TAZO is shown in Table 4. The % RSD for Intraday and Interday precision was found to be 0.454 – 0.64 and 0.74 – 1.01 for TAZO.

Limit of Detection and Limit of Quantification

Limit of Detection was found 3.06 µg/ml for Cefoperazone and 0.35 µg/ml for Tazobactam.

Limit of Quantification was found 9.27 µg/ml for Cefoperazone and 1.06 µg/ml for Tazobactam.

Accuracy (n = 3)

The results of the accuracy study are shown in Table 5 for CEFO and TAZO respectively. The results show that the percentage recoveries for CEFFO and TAZO were found to be in the range of 99.78 - 100.17% and 99.15 - 100.33% respectively

Estimation of Cefoperazone and Tazobactam in formulation (n = 5)

Applicability of proposed method was tested by analyzing Injection formulation (Cegava-Tz). The results are shown in Table 6. The assay results were comparable to labeled value of each drug in injection formulation. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of formulation in industries.

Table No. 4: "Intraday & Interday Precision study of Cefoperazone"

Drug	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ± S.D (n = 3)	% RSD	Mean ± S.D (n = 3)	% RSD
Cefoperazone	37.5	0.010004 ±0.0008944	0.890	0.0101 ± 0.00014142	1.40021
	50	0.01208 ± 0.0001095	0.9068	0.01214 ± 0.00013416	1.1051
	62.5	0.1402 ± 0.00044721	0.318	0.01406 ± 0.0008942	0.6361
Tazobactam	4.6875	0.00698± 0.0004472	0.640	0.007±0.000 7071	1.010
	6.25	0.01004± 0.0005477	0.545	0.01008± 0.000836	0.830
	7.8125	0.01204± 0.00054772	0.454	0.01206± 0.0008944	0.7416

Table No. 5: "Recovery Study Cefoperazone And Tazobactam"

Drug	% of Level	Amount Taken (µg/ml)	Amount Added (µg/ml)	Total Amount Found (µg/ml)	% Recovery ± SD (n = 3)
Cefoperazone	80 %	25	20	44.35	98.56 ± 0.987
	100 %	25	25	48.71	97.43 ± 0.889
	120 %	25	30	54.35	98.82 ± 0.808
Tazobactam	80 %	3.125	2.5	5.37	95.46± 1.231
	100 %	3.125	3.125	6.25	100.2± 0.554
	120 %	3.125	3.75	6.583	95.75± 1.427

Table No. 6: "Analysis of Pharmaceutical Dosage form"

Formulation	Cefoperazone			Tazobactam		
	Amount Labeled (mg)	Amount Found (mg)	% Amount found SD (n = 3)	Amount Labeled (mg)	Amount Found (mg)	% Amount found SD (n = 3)
Cegava-Tz	1000	978.4	97.84 ± 0.8434927	125	125.32	100.256 ±0.52581

Table No. 7: "Optical Regression Characteristics And Validation Parameter"

Parameter	Cefoperazone	Tazobactam
Calibration Range (µg/ml)	25-75	3.125-9.375
Regression Equation	y = 0.00013x + 0.0005	y = 0.00017x + 0.0005
Slop (m)	0.00013	0.00017
Intercept (c)	0.0005	0.0005
Correlation co-efficient (r)	0.989	0.993
Intraday (% RSD, n = 5)	0.318-0.906	0.45-0.64
Interday (% RSD, n = 5)	0.63-1.40	0.74-1.01
Detection limit (µg/ml)	3.06	0.35
Quantitation limit (µg/ml)	9.27	1.06

DISCUSSION

The objective of the proposed work was to develop and validate novel analytical method for simultaneous estimation of Cefoperazone and Tazobactam in pharmaceutical formulations according to ICH guidelines. It has advantage that it eliminates the spectral interference from one of the two drugs while estimating the other drug by selecting zero crossing point in the derivative spectra of each drug at selected wavelength. The ZCPs for CEFO and TAZO were found to be 320 nm and 232.8 nm respectively. Similarly % RSD from the interday precision data were found be found to be 0.63 – 1.40 % for CEFO and 0.74 – 1.01 % for TAZO. The LOD for CEFO and TAZO was found to be 3.066 µg/ml and 0.35 µg/ml respectively.

Results of the validation of the above method indicate that the method was linear in the range of 25-75 µg/ml for CEFO and 3.125- 9.75 µg/ml for TAZO. The % recoveries for CEFO and TAZO obtained in the accuracy study were 97.43 – 98.82 % and 95.46 - 100.2% respectively. The results of the precision study indicate that the proposed method showed good repeatability for CEFO and TAZO with % RSD of 0.938 % and 0.645 % respectively.

The % RSD for Intraday precision was found to be 0.318 – 0.906 % for CEFO and 0.454 – 0.64 % for TAZO. Similarly LOQ for CEFO and TAZO was found to be 9.27 µg/ml and 1.06 µg/ml respectively. The % assay results of 97.84 % for CEFO and 100.25 % for TAZO indicate that the developed method was successfully utilized for the estimation of CEFO and TAZO in their formulation.

CONCLUSION

The proposed method was found to be reproducible and reliable for simultaneous determination of Cefoperazone and Tazobactam in injection dosage form labelled Cegava-Tz. Results are in good agreement with claim which indicates there is no interference of routinely used excipients. The proposed Derivative spectroscopy method was easily and conveniently applied for determination of Cefoperazone and Tazobactam from combined dosage form for regular monitoring, pharmaceutical manufacturing and research. The percentage of Cefoperazone and Tazobactam was found to be satisfactory, which is comparable with the corresponding label claim.

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