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UV spectrophotometric method development and validation for simultaneous determination of cefixime trihydrate and cloxacillin sodium in combined tablet dosage form

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Abstract
A simple, sensitive, accurate and precise UV - spectrophotometric isobastic point method for simultaneous estimation of Cefixime trihydrate and Cloxacillin sodium in tablet dosage form has been developed and validated for accuracy, precision, ruggedness, linearity and range. The wavelengths selected for estimation of drugs were 241.8 nm (isobastic point at which both drug exhibit equal absorbance) and 290.0 nm (λ_{max} of Cefixime trihydrate). Linearity for Cefixime trihydrate and Cloxacillin sodium were 2-20 µg/ml and 5-30 µg/ml respectively. The method gives results for high accuracy and high recovery of 99.76±0.08 and 99.38±0.22 for Cefixime trihydrate and Cloxacillin sodium respectively. % R.S.D. values for marketed formulation analysis were found to be less than 2 which indicated good precision and reproducibility of the method. The method was found to be simple, sensitive, accurate and precise.

Keywords: cefixime trihydrate, Cloxacillin sodium, Isobastic point method

Introduction
Cefixime is an orally third generation cephalosporin antibiotics. Chemically it is a 5-Thia-1- azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, 7- [(2 amino-4thiazolyl) {(carboxymethoxy) imino]acetyle}amino]3ethynyl-8-oxo-trihydrate [1, 3]. Cefixime clinically used in the treatment of susceptible infections including gonorrhoea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary tract infections [3, 5]. It is soluble in methanol and 0.1M NaOH, insoluble water and 0.1M HCL.

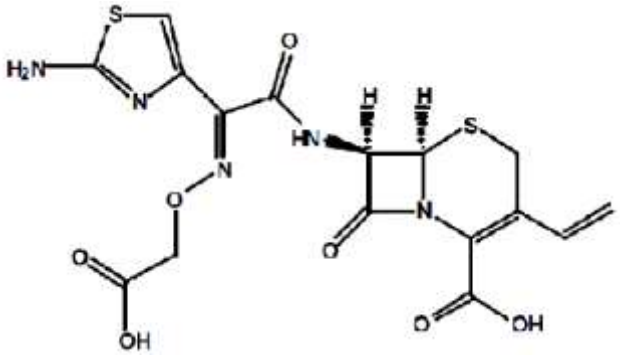

Fig 1: Chemical structure of Cefixime [6, 7]

Cloxacillin, chemically monosodium (2*S*, 5R, 6R)-6-{[3-(2-chlorophenyl)-5-methyl-oxazole-4-carbonyl] amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylicacid is a semisynthetic antibiotic in the same class as penicillin. Cloxacillin is a semisynthetic antibiotic in the same class as penicillin. Cloxacillin is used against staphylococci that produce b-lactamase. This drug has a weaker antibacterial activity than benzyl penicillin, and is devoid of serious toxicity except for allergic reactions. It used against staphylococci that produce *beta*-lactamase [8, 10].

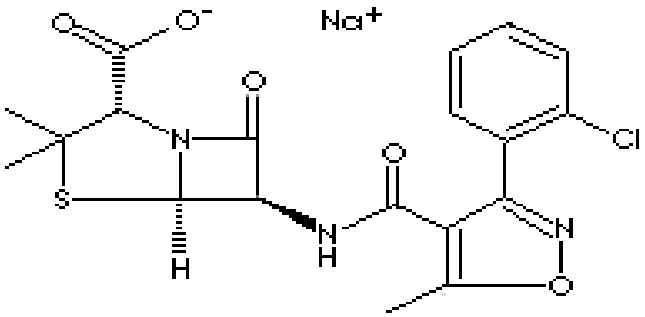

Fig 2: Chemical structure of Cloxacillin [10]

Materials and Method
Chemicals, Instrument and reagents
Cefixime trihydrate and Cloxacillin sodium were obtained as a gift sample from Leben Pharma Pvt. Ltd., Akola, Maharashtra. Combined dose tablet formulation Ceftas-Cl containing Cefixime trihydrate (20mg) and Cloxacillin sodium (50mg) manufactured by Intas Ltd, was purchased from local market. Methanol was used to prepare all solutions. Instrument Shimadzu UV-1800, UV-Visible double beam Spectrophotometer with matching pair of 1 cm quartz cuvettes (Shimadzu Corporation, Kyoto, Japan). The spectral bandwidth is 0.5 nm using UV Probe 2.32 software. Weighing was done on electronic single pan weighing balance (Make: Shimadzu Model: AX 200).

Procedure
Preparation of standard stock solutions
Accurately weighed about 10 mg of CEF and CLOX was transferred to 100.0 ml volumetric flask and 50.0 ml methanol was added to dissolve the drug and diluted up to the mark with methanol, to get 100µg/ml of CEF and 100µg/ml of CLOX in separate volumetric flask.

Preparation of sample solutions

Twenty tablets were weighed and crushed into fine powdered. An accurately weighed quantity of tablet powder equivalent to 10 mg of Cefixime trihydrate and Cloxacillin sodium was transferred to 100 mL volumetric flask and dissolved by sonication with sufficient quantity of methanol and then volume was made up to mark with methanol. The solution was then filtered through wattmann filter paper no.41. The sample solutions of Cefixime trihydrate and Cloxacillin sodium having concentration range of 2-20 µg/mL and 5-30 µg/mL were prepared. The absorbance of the resulting solution was measured at 290.0 nm and 220.0 nm against solvent blank.

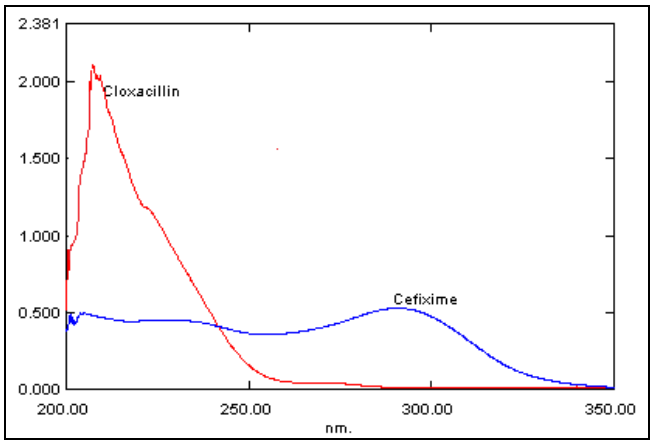

Fig 1: Overlain spectra of CEF (6 µg/ml) and CLOX (15 µg/ml).

From the overlain spectrum (figure1) the wavelengths selected for estimation of drugs were 290.0 nm as detecting wavelength for CEF and 220.0 nm as detecting wavelength for CLOX. Both these drugs obeyed Beers law individually and in mixture within concentration range of 2-30 µg/ml for CEF and 5-30 µg/ml for CLOX. The absorptivity values (A 1%, 1 cm) are recorded in Table 1. The sample absorbance, absorptivity and corrected absorbance were determined and finally the concentration of each drug was calculated using following equation C=A/A (1%, 1 cm) where C, A, A (1%, 1 cm) are the concentration, absorbance and absorptivity at selected wavelengths for selected drugs.

Table 1: (A 1%, 1 cm) values of CEF and CLOX at selected wavelengths

Sr no	CLOX	CLOX	
	220. nm	290.0 nm	220.0 nm
1	276	450	165
2	334	492.5	302.5
3	356.6	523.3	348.3
4	369	508.7	357.5
5	377.6	512	368
Mean S.D.	352.4 2.06203	503.3 0.46043	332.7 0.48476

Application of proposed method to marketed formulation

20 tablets were accurately weighed; average weight was calculated, finely powdered and mixed thoroughly. An accurately weighed capsule powder equivalent to about 2 mg of CEF was transferred to 25 ml volumetric flask. The contents were shaken for 15 minutes with methanol and volume was adjusted up to the mark with same. The solution was filtered through Whatman No.1 filter paper. An accurately measured 1 ml portion of filtrate was further diluted to 10 ml with Methanol. The absorbance of the above solution was then measured at 290 nm and 220 nm in 1 cm cell against methanol as blank. Five replicate estimations were done in similar way. The contents of CEF and CLOX were calculated and % label claim was determined. Observations and results of estimation of CEF and CLOX in marketed formulation analysis are given in Table 2.

Table 2: Observations and results of marketed formulation analysis

Sr. no.	Weight of tablet content(gm)	Absorbance		% Label claim	
		290.0 nm	220.0 nm	CEF	CLOX
1	0.00640	0.303	0.727	101.56	101.6
2	0.00650	0.295	0.722	98.33	99.46
3	0.00654	0.298	0.724	100.54	101.50
Mean				100.14	100.85
S.D.				1.2774	1.1444
R.S.D.				0.01296	0.01154

Validation

Validation of proposed method was carried out for the following parameter as per ICH/USP 16 guidelines. Accuracy of the proposed method was ascertained on the basis of recovery studies, performed by standard addition method. Accurately weighed quantities of preanalysed tablet content equivalent to 2 mg was taken in series of 25 ml volumetric flask and to them known amount of CEF and CLOX were added at different concentration levels so as to produce solutions containing 80%, 100% and 120% of the label claim. Percentage recovery was calculated. The results are shown in Table 3. Precision of the above method was ascertained by replicate estimation of drugs for marketed formulation. The results are shown in Table 2.

Table 3: Observations, results and statistical data for recovery studies

Sr. No.	Wt of tablet powder(gm)	Amount added µg/ml		Absorbance		% Recovery	
		CEF	CLOX	290.0 nm	220.0 nm	CEF	CLOX
1	0.00648	4.8	12	0.306	0.724	101.03	100.97
2	0.00644	6	15	0.303	0.720	99.23	99.40
3	0.00650	7.2	18	0.308	0.725	100.86	101.04
Mean						100.37	100.47
S.D.						0.08145	0.2212
R.S. D						0.0008	0.00223

The ruggedness of the method was studied under three different parameters. Intraday variation (samples were analysed at different times on the same day), Interday variation (samples were analysed at three different days 1st, 3rd and 5th day) and different analyst (samples were analysed by three different analysts as per the proposed method). The results are shown in Table 4.

Table 4: results of Ruggedness studies of proposed method

Parameters	CEF* (Mean±SD.)	CLOX* (Mean±SD.)
Intraday	98.55±1.44	100.17±1.43
Interday	97.10±1.69	98.93±1.43
Different analyst	98.81±1.29	99.52±0.8137

*Results are mean of three determinations, S.D. is standard deviation

For linearity and range capsule powder in the range of 80%-120% of test concentration of label claim was taken. The absorbance of final solutions were read at 253.2 nm and 288.8 nm and a graph was plotted as % test concentration Vs absorbance. CEF and CLOX were found to be linear in the range of the test concentration.

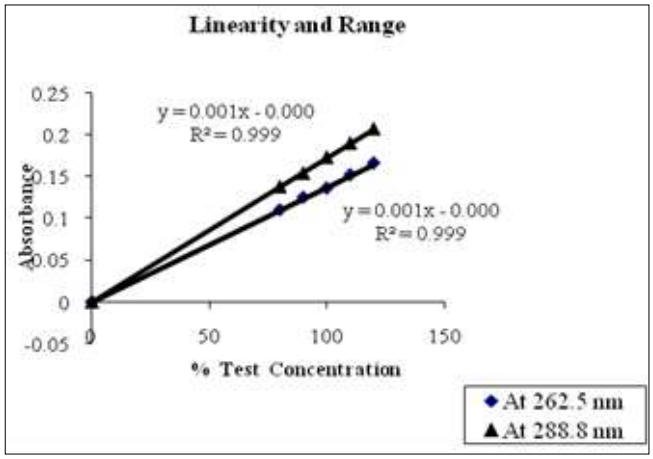

Fig 2: Plot of linearity and range

Results and Discussion

An accurate, rapid, sensitive and economic method for the simultaneous analysis of CEF and CLOX was developed. Accuracy of the proposed method was ascertained by recovery studies. % recoveries with standard deviation were found to be 99.76±0.08 and 99.38±0.22 for CEF and CLOX respectively. The proposed method was also successfully applied to pharmaceutical formulation. No interference was observed from the pharmaceutical adjuvants. The results of marketed formulation analysis were in good agreement with labelled amounts indicating high degree of precision

Conclusion

The applicability for the proposed method for quantitative estimation of CEF and CLOX in commercial formulation gave accurate and precise results. Due to high sensitivity and simple sample preparation can be the part of undergraduate curriculum. Moreover, spectrophotometric methods have obvious advantage over sophisticated instrumental analysis. Hence simple, economical and less time-consuming spectrophotometric methods always have a role in routine pharmaceutical analysis.

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