AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Development and Validation of Analytical Methods for Simultaneous Estimation of Cefixime and Levofloxacin in Pharmaceutical Dosage form

Vishal Bhatt*[1], Chosala Sahdev[1], Darji Suresh[1], Hiren Kadikar[1]

1.Arihant school of pharmacy & BRI, uvarsad square,S. G. Highway, Adalaj, Dist. Gandhinagar-382421, Gujarat

ABSTRACT

Accurate, precise, rapid and economical first order derivative spectroscopic method was developed and validated for the estimation of Cefixime and levofloxacin in tablet. The wavelengths selected for quantitation were 289.45 nm for levofloxacin (zero cross for cefixime) and 317.0 nm for cefixime (zero cross for levofloxacin). Linearity for detector response was observed in the concentration range of 2-12 µg/ml for both Cefixime and Levofloxacin using methanol as a solvent with correlation coefficient 0.991 and 0.993 respectively. The proposed method was successfully applied for the simultaneous estimation of both drugs in tablet.

Keywords: Cefixime, levofloxacin, UV specroscopy, method validation, tablet

*Corresponding Author Email: vrb21690@gmail.com

Received 14 December 2012, Accepted 22 December 2012

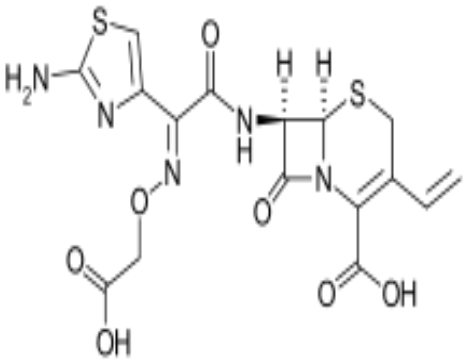
Please cite this article in press as Bhatt V *et al.*, Development and Validation of Analytical Methods for Simultaneous Estimation of Cefixime and Levofloxacin in Pharmaceutical Dosage form. American Journal of PharmTech Research 2013.

INTRODUCTION

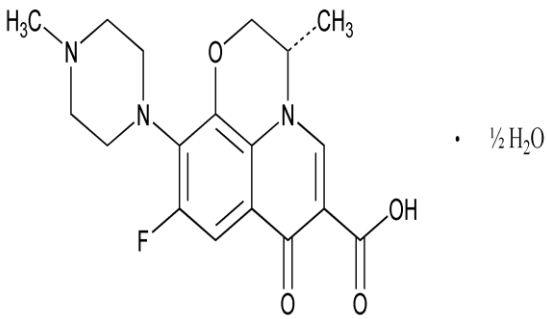
Cefixime, an antibiotic, is (6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-[(carboxymethoxy)imino]acetamido]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, a third-generation cephalosporin like ceftriaxone and cefotaxime. Cefixime is highly stable in the presence of beta-lactamase enzymes. As a result, many organisms resistant to penicillins and some cephalosporins due to the presence of beta-lactamases, may be susceptible to cefixime. The antibacterial effect of cefixime results from inhibition of mucopeptide synthesis in the bacterial cell wall. Cefixime is used in the treatment of the following infections when caused by susceptible strains of the designated microorganisms: (1) uncomplicated urinary tract infections caused by Escherichia coli and Proteus mirabilis, (2) otitis media caused by Haemophilus influenzae (beta-lactamase positive and negative strains), Moraxella catarrhalis (most of which are beta-lactamase positive), and S. pyogenes, (3) pharyngitis and tonsillitis caused by S. pyogenes, (4) acute bronchitis and acute exacerbations of chronic bronchitis caused by Streptococcus pneumoniae and Haemophilus influenzae (beta-lactamase positive and negative strains), and (5) uncomplicated gonorrhea (cervical/urethral) caused by Neisseria gonorrhoeae (penicillinase- and non-penicillinase-producing strains).

Levofloxacin is a (2S)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-zatricyclo[7.3.1.0^{5,13}]trideca-5(13),6,8,11-tetraene-11-carboxylic acid.

It is a synthetic fluoroquinolone (fluoroquinolones) antibacterial agent that inhibits the supercoiling activity of bacterial DNA gyrase, halting DNA replication and is used for the treatment of bacterial conjunctivitis caused by susceptible strains of the following organisms: Corynebacterium species, Staphylococus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Streptococcus (Groups C/F/G), Viridans group streptococci, Acinetobacter lwoffii, Haemophilus influenzae, Serratia marcescens.



Cefixime



Levofloxacin

Based on my current and ongoing referencing work, till date, I have not come across any official and reported analytical methods for simultaneous estimation of both the drugs in their combined dosage form. Therefore, the objective is to develop a spectroscopic for simultaneous estimation of Cefixime and Levofloxacin in their formulation and to validate the developed method.

MATERIALS AND METHOD:

Apparatus and Instrument

Double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path. Electronic analytical balance (AUX-220), Shimadzu, Japan. Volumetric flask – 10, 50, 100 ml (Durasil).Pipettes – 1, 2, 5, 10 ml

Chemicals and Reagents

Methanol AR (Chemco®, Chemdys Corporation, Rajkot),Cefixime (Gift sample from Sohm labs, Ahmedabad),Levofloxacin (Gift sample from Weast coast pharma, Ahmedabad and Troikaa pharma), CEFI-L Tablet (Abbott pharmaceutical)

Derivative Conditions

Mode : Spectrum, Scan speed : Medium, Wavelength range : 200-400 nm, Derivative order : 1

The derivative spectra were recorded by using digital differentiation (Convolution method) with a derivative wavelength difference (Δλ (N)) of 2 nm in the range of 200-400 nm.

Selection of Solvents

From literature review of the both drugs, it is known that both drugs are soluble in methanol ,so analysis was carried out by using methanol as a solvent.

Preparation of standard stock solution of cefixime

Accurately weighed quantity of 10 mg cefixime was transferred into 10 ml volumetric flask, dissolved and diluted up to mark with solvent Methanol to produce a stock solution (1000µg/ml).

Preparation of standard stock solution of levofloxacin

Accurately weighed quantity of 10 mg was transferred into 10 ml volumetric flask, dissolved and diluted up to mark with solvent Methanol to produce a stock solution (1000µg/ml)

Preparation of working standard solution of cefixime

100 µg/ml of cefixime solution was prepared by diluting 1 ml of stock solution to 10 ml with solvent Methanol. 10 µg/ml of cefixime solution was prepared by taking 5 ml aliquot of resulting solution and dilute up to 50 ml with solvent Methanol.

Preparation of working standard solution of levofloxacin

100 µg/ml of levofloxacin solution was prepared by diluting 1 ml of stock solution to 10 ml with solvent Methanol. 10 µg/ml of Levofloxacin solution was prepared by taking 5 ml aliquot of resulting solution and dilute up to 50 ml with solvent Methanol.

Preparation of Calibration Curve

Calibration curve for cefixime consisted of different concentrations of standard cefixime solution ranging from 2-12 µg/ml. Calibration curve for levofloxacin consisted of different concentrations of standard Levofloxacin solution ranging from 2-12 µg/ml. The solutions were prepared by pipetting out 2.0, 4.0, 6.0, 8.0 ml from the working standard solution of cefixime (10 µg/ml) and from the working standard solution of levofloxacin (10 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted up to mark with Solvent Methanol. It gives 2.0-8.0 µg/ml of both drugs. 10 µg/ml was the working standard solution for both drugs. 12 µg/ml for both drugs were prepared by pipetting out 1.2 ml from working standard solution of 100 µg/ml into10 ml volumetric flasks and the volume was adjusted up to mark with Solvent Methanol.

Selection of Wavelength for Simultaneous Estimation of Cefixime and Levofloxacin

Zero order overlain spectra for cefixime and levofloxacin were taken in the range of 2-12 µg/ml (2, 4, 6, 8, 10, 12 µg/ml). Each of the solution was scanned between 200 – 400 nm at a medium scanning speed. It showed wavelength maxima at 289.45 and 295.0 nm respectively(figure : 3,4) All the Zero order overlain spectra were then converted to their respective 1st order derivative spectra using the inbuilt software. First order derivative spectrum for cefixime showed two zero crossing points : 289.45 nm and 340.00nm to 400nm. The wavelength selected for estimation of levofloxacin was 289.45 nm (figure : 5) similarly, first order derivative spectrum for levofloxacin was taken and it showed three zero crossing points; 295.0 nm, 317.0 nm, and 360.0 nm to 400 nm. The wavelength selected for estimation of cefixime was 317.0 nm. Absorbance of each solution was measured at 289.45 nm for Levofloxacin and 317.00 nm for cefixime using first order derivative spectrophotometry. The graph of response verses respective concentration was plotted.

VALIDATION OF ANALYTICAL METHODS

Validation of analytical procedures is the process of determining the suitability of a given methodology for providing useful analytical data. Validation is the formal and systematic proof that a method complies with the requirements for testing a product when observing defined procedures. Method validation is primarily concerned with identification of the sources of potential errors and quantification of the potential errors in the method.

Analytical parameters considered in the validation of assay

- Accuracy
- Precision
- Specificity
- Limit of Detection
- Limit of Quantitation
- Linearity
- Range
- Ruggedness
- Robustness
- Selectivity

Accuracy

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. Accuracy may often be expressed as percent recovery by the assay of a known amount of analyte added. Accuracy may be determined by applying the method to samples or mixtures of excipients to which known amount of analyte have been added both above and below the normal levels expected in the samples. Accuracy is then calculated from the test results as the percentage of the analyte recovered by the assay. Dosage form assays commonly provide accuracy within 3-5% of the true value. The ICH documents recommend that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e. three concentrations and three replicated of each concentration).

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. This is usually expressed as the standard deviation or the relative standard deviation (coefficient of variation). Precision is a measure of the degree of reproducibility or of the repeatability of the analytical method under normal operating circumstances.

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time.

Determination of Repeatability

Repeatability can be defined as the precision of the procedure when repeated by same analyst under the same operating conditions (same reagents, equipments, settings and laboratory) over a short interval of time.

It is normally expected that at least six replicates be carried out and a table showing each individual result provided from which the mean, standard deviation and co-efficient of variation should be calculated for set of n values. The RSD values are important for showing degree of variation expected when the analytical procedure is repeated several times in a standard situation. (RSD below 1% for bulk drugs, RSD below 2% for assays in finished product).

The ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e. three concentrations and three replicates of each concentration or using a minimum of six determinations at 100% of the test concentration).

Determination of reproducibility

Reproducibility means the precision of the procedure when it is carried out under different conditions-usually in different laboratories-on separate, identical samples taken from the same homogenous batch of material. Comparisons of results obtained by different analysts, by the use of different equipments, or by carrying out the analysis at different times can also provide valuable information.

Linearity and Range

The linearity of an analytical method is its ability to elicit test results that are directly (or by a well defined mathematical transformation) proportional to the analyte concentration in samples within a given range. Linearity is usually expressed in terms of the variance around the slope of regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte. The linear range of detectability that obeys Beer‟s law is dependent on the compound analyzed and the detector used. The working sample concentration and samples tested for accuracy should be in the linear range. The claim that the method is linear is to be justified with additional mention of zero intercept by processing data by linear least square regression. Data is processed by linear least square regression declaring the regression co-efficient and b of the linear equation $y = ax + b$ together with the correlation coefficient of determination r. For the method to be linear the r value should be close to1.

The range of an analytical method is the interval between the upper and lower levels of the analyte (including these levels) that have been demonstrated to be determined with precision,

accuracy and linearity using the method as written.

Limit of Detection and Limit of Quantitation

Limit of detection

The limit of detection is the parameter of limit tests. It is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions. The limit test thus merely substantiates that the analyte concentration is above or below a certain level.

The determination of the limit of detection of instrumental procedures is carried out by determining the signal-to-noise ratio by comparing test results from the samples with known concentration of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted.

The signal-to-noise ratio is determined by dividing the base peak by the standard deviation of all data points below a set threshold. Limit of detection is calculated by taking the concentration of the peak of interest divided by three times the signal-to-noise ratio.

For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (S_a) which may be related to LOD and the slope of the calibration curve, b, by

$$LOD = 3\ S_a / b$$

Limit of quantitation

Limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices such as impurities in bulk drugs and degradation products in finished pharmaceuticals. The limit of quantitation is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied.

It is measured by analyzing samples containing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision are attainable. Where the final assessment are based on an instrumental reading, the magnitude of background response analysed by a number of blank samples and calculating the standard deviation of this response. The standard deviation multiplied by a factor (usually 10) provides an estimate of the limit of quantitation. In many cases, the limit of quantitation is approximately twice the limit of detection.

Selectivity and Specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix.

If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. On the other hand, if the method determines or measures quantitatively the component of interest in the sample matrix without separation, it is said to be specific.

Hence one basic difference in the selectivity and specificity is that, while the former is restricted to qualitative detection of the components of a sample, the latter means quantitative measurement of one or more analyte.

Selectivity may be expressed in terms of the bias of the assay results obtained when the procedure is applied to the analyte in the presence of expected levels of other components, compared to the results obtained on the same analyte without added substances. When the other components are all known and available, selectivity may be determined by comparing the test results obtained on the analyte with and without the addition of the potentially interfering materials. When such components are either unidentified or unavailable, a measure of selectivity can often be obtained by determining the recovery of a standard addition of pure analyte to a material containing a constant level of the other components.

Robustness and Ruggedness

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The determination of robustness requires that method characteristics are assessed when one or more operating parameter is varied.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory. Ruggedness is normally expressed as the lack of the influence on the test results of operational and environmental variables of the analytical method.

For the determination of ruggedness, the degree of reproducibility of test result is determined as

function of the assay variable. This reproducibility may be compared to the precision of the assay under normal condition to obtain a measure of the ruggedness of the analytical method.

RESULT AND DISCUSSION:

Linearity and Range

Calibration curve of cefixime

The calibration curves for cefixime and levofloxacin were prepared and responses were measured at 289 nm (ZCP of cefixime) and 317 nm (ZCP of levofloxacin). It was found that Lambert-Beer's law was followed in the concentration ranges of 2-12µg/ml cefixime and levofloxacin . The data for responses of cefixime at 317 nm and responses of levofloxacin at 289 nm are shown in Table 1 and 2 respectively. The linearity curves for cefixime and levofloxacin are shown in Figure. 1& 2 respectively. The straight line equations and correlation coefficient for CINNA and DIMEN are shown in Table (2,4).

Table 1: Data of Response vs concentration of cefixime

Sr. No	Conc. (µg/ml)	Response |Mean| ± S.D	%RSD
1	2	|0.0012| ± 0.00004	0.64
2	4	|0.0038| ± 0.00008	0.86
3	6	|0.0054| ± 0.00008	0.58
4	8	|0.0080| ± 0.00008	0.43
5	10	|0.0092| ± 0.00050	1.97
6	12	|0.0113| ± 0.00050	1.68

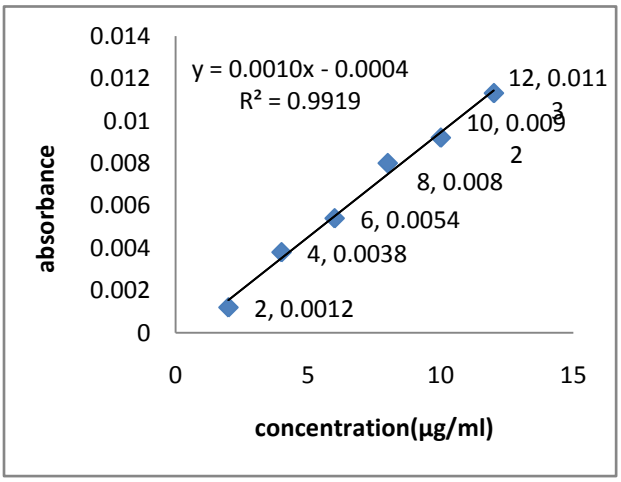


Figure. 1: Calibration curve of cefixime (2-12µg/ml).

Table 2: Data of regression analysis.

Linearity	y = 0.0010x - 0.0004
Regression co-efficient	0.9919

Table 3: Data of Response vs concentration of levofloxacin

Sr. No	Conc (µg/ml)	Response |Mean| ± S.D	%RSD
1	2	|0.0068| ± 0.00004	.65
2	4	|0.0113| ± 0.00008	.78
3	6	|0.0146| ± 0.00008	.55
4	8	|0.0173| ± 0.00008	.46
5	10	|0.0218| ± 0.00020	.23
6	12	|0.0266| ± 0.00050	.43

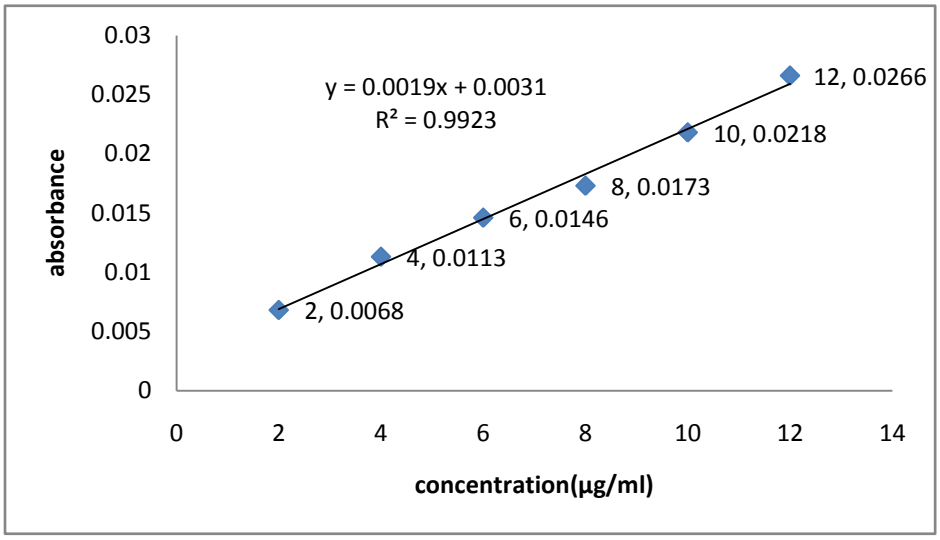


Figure. 2: Calibration curve of levofloxacin (2-12µg/ml).

Table 4: Data of regression analysis.

Linearity	y = 0.0019x + 0.0031
Regression co-efficient	R² = 0.9923

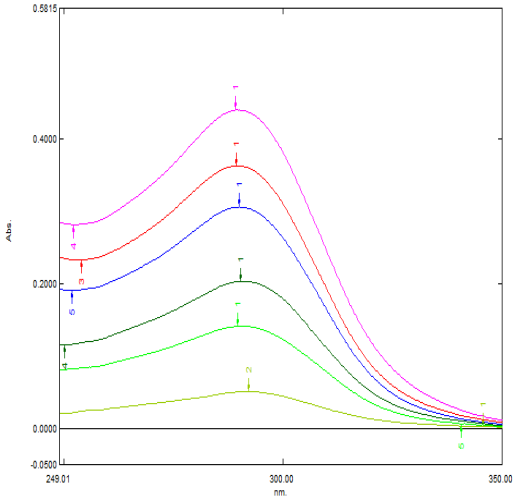


Figure: 3 Overlay Zero order spectra of Cefixime (2-12µg/ml).

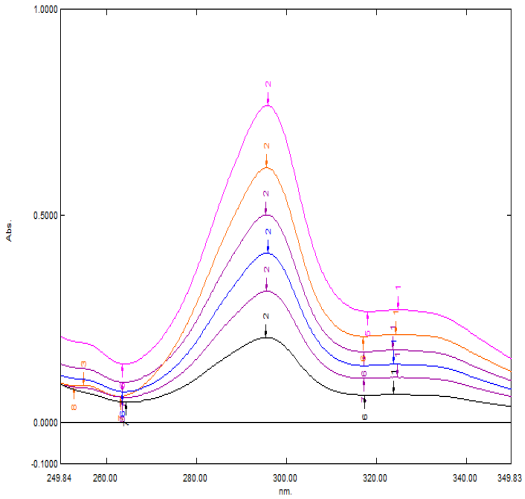


Figure: 4 overlay zero order spectra of Levofloxacin(2-12µg/ml).

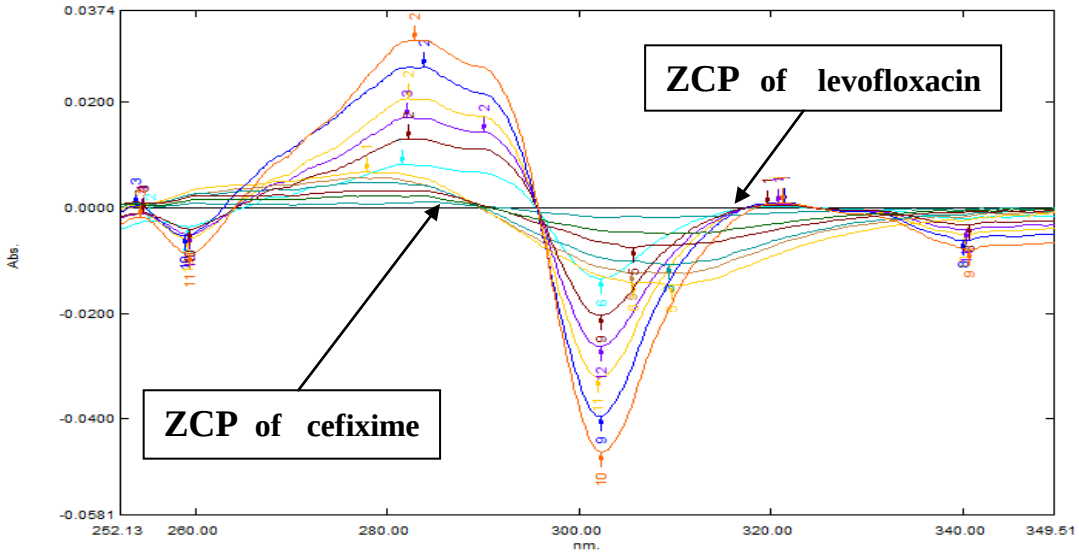


Figure:5 Overlay first order spectra of Cefixime (2-12 µg/ml) and Levofloxacin(2-12 µg/ml)

Accuracy (n = 3)

The results of the accuracy study are shown in Table 5 and 6 for cefixime and levofloxacin respectively. The results show that the percentage recoveries for cefixime and levofloxacin were found to be in the range of 99.78 - 100.17% and 99.15 - 100.33% respectively.

Table 5: Determination of Accuracy of Cefixime (n = 3)

Amt of Cefixime present (mg)	% Amt of std Cefixime added	Total Amt of cefixime present (mg)	Amount recovered mean (mg)	SD	% Recovery
20	0%	20	19.79	0.0346	99.95
20	50%	30	29.95	0.1106	99.83
20	100%	40	40.07	0.1625	100.17
20	150%	50	49.89	0.0346	99.78

Table 6: Determination of Accuracy of levofloxacin (n = 3)

Amt of levo present (mg)	% Amt of std levo added	Total Amt of levo present (mg)	Amount recovered mean (mg)	SD	% Recovery
25	0%	25	24.68	0.0251	99.15
25	50%	37.5	37.70	0.0351	100.33
25	100%	50	50.11	0.0458	100.12
25	150%	62.5	62.16	0.0404	99.76

Precision:

Repeatability (n = 6):

The repeatability data for cefixime and levofloxacin are shown in table 7. the % cv was found to be 0.23% for cefixime and 0.22% for levofloxacin.

Table 7: Repeatability data for cefixime and levofloxacin (n =6)

Conc. of Cefixime (µg/ml)	Response	Conc. of levofloxacin (µg/ml)	Response
	0.0043		0.0124
	0.0041		0.0122
	0.0042		0.0120
	0.0044		0.0126
4	0.0043	5	0.0125
	0.0042		0.0124
Mean	0.0042	**Mean**	0.0123
SD	0.0000097	**SD**	0.0000281
% CV	0.23	**% CV**	.22

Intraday precision (n = 3):

The data for intraday precision for cefixime and levofloxacin is shown in table 8. The % cv for intraday precision was found to be 0.35 - 0.46 for cefixime and 0.26 – 1.02 for levofloxacin.

Table 8: Data for Intraday Precision for cefixime and levofloxacin (n = 3)

CEFIXIME			LEVOFLOXACIN		
Conc. (µg/ml)	**Mean response ± SD**	**% CV**	**Conc. (µg/ml)**	**Mean response ± SD**	**% CV**
04	0.0043 ± 0.0000152	0.35	04	0.0113 ± 0.0000264	0.26
05	0.0048 ± 0.0000200	0.41	05	0.0128 ± 0.0000737	0.57
06	0.0054 ± 0.0000251	0.46	06	0.0146 ± 0.000149	1.02

Interday precision (n = 3):

The data for interday precision for cefixime and levofloxacin is shown in table 9. the % cv for intraday precision was found to be 0.92 – 1.25 for cefixime and 0.53 – 1.34 for levofloxacin.

Table 9: Data for Interday Precision for cefixime and levofloxacin (n = 3)

CEFIXIME			LEVOFLOXACIN		
Conc. (µg/ml)	**Mean response ± SD**	**% CV**	**Conc. (µg/ml)**	**Mean response ± SD**	**% CV**
04	0.0040 ± 0.00005	1.25	04	0.0105 ± 0.0000550	0.53
05	0.0045 ± 0.0000416	0.92	05	0.0124 ± 0.000133	1.07
06	0.0051 ± 0.0000568	1.11	06	0.0140 ± 0.000188	1.34

LOD and LOQ:

LOD for cefixime and levofloxacin was found to be 0.3943 µg/ml and 0.9211 µg/ml respectively. similarly LOQ for cefixime and levofloxacin was found to be 1.1951 µg/ml and 2.7914 µg/ml respectively.

Estimation of Cefixime and Levofloxacin in their combined dosage form by UV-spectroscopic method (n = 5):

Applicability of the proposed method was tested by analyzing the commercially available tablet formulation CEFI-L (fig-6). The assay data are shown in the Table 10.The assay results

were comparable to labeled value of each drug in tablet dosage form. These results indicate that the developed method is accurate, precise, cefiximeple and rapid. It can be used in the routine quality control of dosage form in industries.

Table 10: Analysis of marketed formulation (n = 5)

CEFIXIME		LEVOFLOXACIN	
Amt Present in tablet (mg)	**Amt found in assay (mg)**	**Amt Present in tablet (mg)**	**Amt found in assay (mg)**
400	400.66	500	500.64
	400.80		500.61
	400.73		500.68
	400.86		500.64
	400.73		500.76
MEAN ± SD	400.75 ± 0.0763	MEAN ± SD	500.66 ± 0.0581
% CV	0.3864	% CV	0.1465
% Labeled Claim	98.78 %	% Labeled	99.16 %

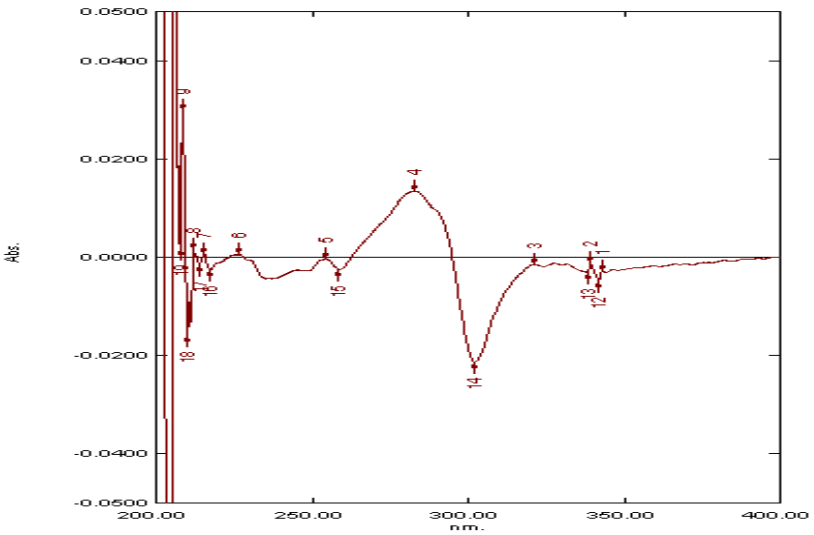


Figure: 6 First order specra of tablet

Summary of validation parameters:

All the validation parameters are shown in Table 11

Table 11: Summary of validation parameters

PARAMETER	CEFIXIME	LEVOFLOXACIN
Linearity	2-12 µg/ml	2-12 µg/ml
Accuracy (% Recovery) (n=3)	99.78-100.17 %	99.15-100.33 %
Precision (%CV)		
Repeatability (n=6)	0.23	0.22
Intraday (n=3)	0.35 - 0.45	0.26 -1.02
Interday (n=3)	0.92 – 1.25	0.33 – 1.34
LOD (µg/ml)	0.3943	0.9211
LOQ (µg/ml)	1.1951	2.7914

CONCLUSION:

A simple and specific first order derivative UV spectrophotometric method has been developed and validated for simultaneous estimation of cefixime and levofloxacin. The wavelength of estimation was 317 nm (zero crossing point of levofloxacin) for cefixime and 289.45 nm (zero crossing point of cefixime) for levofloxacin. The method was validated for linearity, precision, accuracy, LOD and LOQ.

The method was applied to the marketed tablet. assay value of cefixime was found to be 98.78 of % labeled claim and assay value of levofloxacin was found to be 99.16 of % labeled claim for tablet. The linearity of developed method for simultaneous estimation was achieved in the range of 2-12 µg/ml (r^2=0.999) for cefixime and 2-12 µg/ml (r^2=0.9999) for levofloxacin. The recovery was in the range of 99.78-100.17 % for cefixime and 99.15-100.33 % for levofloxacin. The method was found to be accurate, precise, specific, selective, and repeatable. Limit of quantitation for cefixime and levofloxacin was found to be 1.1951µg/ml and 2.7914µg/ml respectively.

REFERENCES:

1. Rang HP., Dale MM., Ritter JM., and Flower RJ. Rang and Dale's Pharmacology, 6th Ed: Churchill Livingstone, Elsevier Science Ltd.; 2007: 275-286.
2. Tripathi KD. Essential of Medical Pharmacology, 5th Edn, New Delhi: Jaypee Brothers Medical Publishers; 2003: 121, 197, 435, 665.
3. Beckett AH. Practical pharmaceutical chemistry; 3rd Edn; Vol 2: 248.
4. Ahuja S. Modern Pharmaceutical Analysis: Separation Science and Technology, 3rd Edn: 1st Indian Reprint, Academic Press; 2005: 98-110.
5. Gertz C. HPLC Tips and Tricks, Alden press, Oxford; 1990: 1-84.
6. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use. Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1) 2005.
7. Budavari S. "The Merck Index" An encyclopedia of chemicals, drugs and biological, 14th Edn; Merck Research Laboratories; 2004:143-144, 668.
8. Colin D. Therapeutic drugs, 2nd Edn; Vol 1, Churchill Livingstone; 1999: A228-A232.
9. Parfit K. The complete drug reference, 35th Edn London, UK: Pharmaceutical press; Vol 1, 2007: 1034-1037.

10. Cefixime, “Introduction”, June 2011, http://en.wikipedia.org/wiki/cefixime
11. Cefixime, “Pharmacology”, June 2011, http://www.drugs.com
12. Cefixime, “Description”, June 2011, http://www.rxlist.com/cgi/generic/asa.htm
13. Levofloxacin, “Introduction”, June 2011, http://www.drugs.com/cdi/levofloxacin.html
14. Levofloxacin, “Description”, June 2011, http://en.wikipedia.org/wiki/levofloxacin
15. Levofloxacin, “Description”, June 2011, http://www.rxlist.com/cgi/generic/asa.htm
16. United States Pharmacopoeia-30, Validation of Compendial Methods, Rockville MD USA, United States Pharmacopeial Convention Inc; 2007, pp 411.
17. European Pharmacopoeial forum - 5.0, pp-1211
18. The Indian Pharmacopoeia, Govt. of India, Ministry of Health & Family Welfare Indian Pharmacopoeia commission, Ghaziabad, 2010, Vol-II, pp 1012.
19. Japanese Pharmacopoeia 2009, Vol I-II; Monograph: Medicinal and Pharmaceutical substances, pp 101.
20. The British Pharmacopoeia, The Department of health, London: The Stationary Office, 2011, Vol-II, pp 309.
21. Kishore L, Nair A and Kaur N. Kinetic spectrophotometric method for the estimation of cefixime in pharmaceutical formulations. Der Pharma Chemica 2011; 3(4): 279-91.
22. Pillai SN, Sahu SK, and Keskar NP. Spectrophotometric estimation of cefixime and ofloxacin from tablet dosage form. Int. J. of Pharm. & Life Sci. 2011; 2(3): 629-632.
23. Gupta S and Gunturu C. Determination of cefixime and ofloxacin by ratio spectra and zero crossing difference spectrophotometry. Int. J. Pharm. Sci. 2011; 4(3): 118-123.
24. Nanda S and Chomwal R. Spectrophotometric estimation of cefixime and ofloxacin in tablet formulation. J. Chem. Pharm. Res. 2011; 3(5): 705-709.
25. Patel R. Method devlopment and validation of cefixime and moxifloxacin in pharmaceutical dosage form by uv spectrophotometric method. Int. J. Pharma. Res. & Bio-sci. 2012; 1(2): 81-93.
26. Shah V and Raj S. Development and validation of derivative spectroscopic method for simultaneous estimation of cefixime trihydrate and azithromycin dihydrate in combined dosage form. Int. J. Pharm. Sci. Res. 2012; 3(6): 1753-1760.
27. Attimarad M and Nair A. Simultaneous determination of ofloxacin and cefixime by first and ratio first derivative UV spectrophotometry. Int. J. Pharm. Sci. 2011; 2(3): 144-149.
28. Ramesh J, Prakasam K and Cothapalli V. Development and validation of

simultaneous equation method for the estimation of cefixime and ofloxacin in pure and tablet formulation. Int. Res. J. Phar. 2011; 2(8) : 109-111.

29. Pash K, Patil C and Chimkod B. Reverse phase hplc method for the determination of cefixime in pharmaceutical dosage forms. Res. J. Pharm. Bio. Che. Sci. 2010; 1(3): 226.
30. Muhammad I. Development of hplc-uv method for analysis of cefixime in raw materials and in capsule. Jord. Journ.of Pharma. Sci. 2009; 29(1): 53-56.
31. The Indian Pharmacopoeia, Govt. of India, Ministry of Health & Family Welfare, Indian Pharmacopoeia commission, Ghaziabad, 2010, Vol-II, pp 1297.
32. Authorized USP Pending Monograph,Version 2, The United States Pharmacopoeia Convention, Rockville, MD, 2009.
33. Levofloxacin Draft Proposal For The International Pharmacopoeia, September, 2010.
34. Desai V and Ozadheoghene E. A simple uv spectrophotometric method for the determination of levofloxacin in dosage formulations. Trop J Pharm Res. 2011; 10 (1): 75.
35. Shirkhedkar A and Surana S. Quantitative determination of levofloxacin hemihydrate in bulk and tablets by uv-spectrophotometry and first order derivative methods. Pak.J.Pharm. Sci. 2009; 229(3): 301-302.
36. Kassab M. Development and validation of uv spectrophotometric method for determination of levofloxacin in pharmaceutical dosage forms. Quim. Nova. 2007; 33(4): 968-971, 201.
37. Dogra S, Panchru D, Simran S. Development and validation of uv- visible spectroscopic method for the estimation of levofloxacin hemihydrate in bulk and marketed formulation. Inter J. of Inst. Pharm. and Life Sci. 2011; 1(2): 57.
38. Makarand AM, Bhandarkar SD. Development and validation of simultaneous uv spectrophotometric method for the determination of levofloxacin and ambroxol in tablets. Int. J. Chem Tech. Res. 2009; 1(4) : 874.
39. Sen A, Sahoo U. Simultaneous estimation of levofloxacin hemihydrate and ornidazole in tablet dosage form by using simultaneous equations. Int. J. Pharma. Sci. 2012; 3(1): 54-57.
40. Kothekara K, Jayakara B, Khandhar A, Mishra R. Quantitative determination of levofloxacin and ambroxol hydrochloride in pharmaceutical dosage form by reversed-phase high performance liquid chromatography. Eurasian J. Anal. Chem. 2007; 2(1): 23.