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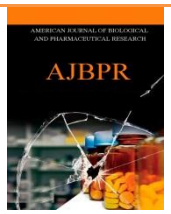

SIMULTANEOUS ESTIMATION OF AZITHROMYCIN AND CEFIXIME IN BULK AND PHARMACEUTICAL FORMULATION BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Azithromycin and Cefixime are both antibiotics drugs. As there is no UV or HPLC method for the simultaneous estimation of Azithromycin and Cefixime, a need was felt to develop the method for the analysis of both drugs simultaneously. This work concerns with the development and validation of a simple, specific and cost effective RP-HPLC method for simultaneous estimation of Azithromycin and Cefixime in bulk. In addition the developed method was applied to the suitable combined tablet dosage form ie., Zifi-AZ. Chromatography was carried on Kromasil C18 column with mobile phase comprising of Phosphate buffer (pH-5): Methanol: Acetonitrile (40:30:30, V/V/V). The flow rate was adjusted to 1ml/min with PDA detection at 260 nm. The retention times of Azithromycin and cefixime were found to be 2.8 min, 3.9 min respectively and other replicate standard system suitability parameters are within the limit and uniform. The different analytical parameters such as accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ) were determined according to the International Conference on Harmonization (ICH) Q2B guidelines. The detector response was linear in the range of 0.5-1.5 mg/ml., 0.4-1.2 mg/ml for Azithromycin and cefixime respectively. The proposed method was successfully applied for the reliable quantification of active pharmaceuticals present in the commercial formulations.

INTRODUCTION

Azithromycin [9-de-oxy-9a-aza-9a-methyl-9a-homoerythromycin] is an azalide, a subclass of macrolide antibiotics [1]. It acts by inhibiting protein synthesis by binding reversibly to the 'P' site of the 50S ribosomal subunit of the bacteria [2, 3]. It is used for adult and pediatricb [4, 5] infections. e.g., respiratory tract infection [6-8] skin, soft tissue infections, otitis media [9], sinusitis, pharyngitis, acute bronchitis, community-acquired pneumonia1, cystic fibrosis [10, 11], tonsillitis [12, 13], anti-inflammatory in COPD patient [14], in P. falciparum malaria with other antimalarial drugs [15], typhoid fever [16, 17].

Cefixime (6R, 7R)-7-[2-(2-amino-4-thiazolyl) glyoxylamido]-8-oxo-3-vinyl-5-1–azabicyclo [4.2.0] oct-2-ene-2-carboxylicacid,7-9z)-[o-carboxymethyl)-oxime] is

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third generation cephalosporin antibiotic. It is under the category of β-lactam antibiotics/cell wall inhibitor. It Acts by inhibiting an enzyme transpeptidase, involved in the building of bacterial cell wall [18]. It is used in lower respiratory tract infections [19-21]. Acute urinary tract infections [21, 22], biliary tract infections [23], sinusitis [24], acute otitis media [25], eptic ulcer [26].

Combination of Cefixime and Azithromycin has a synergistic effect. The effect of Cefixime against neissaria gonorrhoeae can be significantly enhanced in combination with Azithromycin [27]. This Combination is used in treatment of uncomplicated gonococcal urethritis [29], gonorrhea [28], typhoid fever [30-32].

Literature survey reveals that HPLC, RP-HPLC, UV-Visible Spectrophotometry, UPLC [33-41] methods were reported for the estimation of Azithromycin alone or in combination with other drugs except Azithromycin. As per literature survey, no analytical method has been reported for simultaneous estimation of Cefixime and Azithromycin in pharmaceutical dosage forms. Therefore the present research work, our aim is to develop a novel, simple, accurate, sensitive, reproducible, economical analytical method to estimate Azithromycin & Cefixime in their combined dosage form in routine analysis.

MATERIALS AND METHODS Chemicals Reagents

Working standards of pharmaceutical grade Azithromycin & Cefixime were obtained as generous gifts from Dr.Reddy's laboratories (Hyderabad, AP, India) used as such without further purification. The pharmaceutical dosage form used in the study was Zifi-AZ. Methanol (HPLC grade), OPA (AR grade) purchased from Merck specialities Pvt.ltd (Mumbai, India) and double distilled water used for analysis.

Instrumentation and chromatographic condition

Chromatography was carried out on kromasil C18 column with mobile phase comprising of OPA buffer and MeOH in the ratio of 70:30. The flow rate was adjusted to 1ml / min with PDA detection at 292 nm.

Preparation of standard solution

Standard stock solutions of pure drugs were prepared separately by dissolving 10 mg of Azithoromycin in 10ml MeOH and 8mg of Cefixime in 10ml MeOH to get concentrations 1mg/ml and 0.8 mg /ml respectively.

Preparation of sample solution

20 tablets were weighed accurately, powdered and equivalent weight was calculated. The equivalent weight of two tablets were taken and dissolved in 100 ml of MeOH to get the concentration 4 mg/ml of cefixime and 5mg /ml of Azithromycin. From stock solution 2 ml was taken and diluted to 10 ml with MeOH to get concentrations 0.8 mg/ml cefixime and 1mg/ml of Azithromycin.

RESULTS

Validation

The developed method was validated with different analytical parameters such as accuracy, precision, linearity, limit of detection, limit of quantification and robustness according to the international conference on harmonization (ICH) Q2B guidelines.

Precision

Precision of these methods was checked by analyzing the samples at three different time intervals of the same day (intraday precision (table-2)) as well as on different days (interday precision). Robustness for HPLC method was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 mL/min to 0.8 mL/min and 1.2 mL/min while ratio of the mobile phase was changed by ± 1%. Results of the Robustness were shown in table-1.

Recovery studies

To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 50, 100, 150% levels (table-3 and table-4). From the total amount of drug found percentage recovery was calculated.

Linearity

LOD and LOQ

Limit of Detection (LOD) and Limit of quantification (LOQ) were calculated by using the values of slopes and intercepts of the calibration curves for both the drugs. LOD and LOQ values for Azithromycin were found to be 0.89 and 2.99 and for Cefixime 0.71 and 2.39.

Robustness

Method robustness was determined by the small changes in chromatographic conditions like as 0.2ml flow rate and ±5ºc temperature and inject the sample observe the result there were no marked changes compare to other analysis.

RESULTS AND DISCUSSIONS

Retention times of Azithromycin and Cefixime were found to be 2.8 and 3.9 respectively (as shown in Fig. 3). The detector response was linear in the range of 1 to 5 µg/ml for Azithromycin and Cefixime respectively. In the linearity study the regression equation and coefficient of correlation for Azithromycin and Cefixime were found to be ($y = 4838.1x + 1027.3$, $R^2 = 0.9998$), ($y = 4697.5x + 1638.5$, $R^2 = 0.9996$) respectively. Commercial formulations containing Azithromycin and Cefixime were analyzed by the proposed method. A typical chromatogram of marketed formulation is shown in fig. no.3. Six replicate

analysis of formulation were carried out and the mean assay values were found close to 100 %. The tailing factors were <2.0 for both the peaks. The elution order was Azithromycin (RT = 2.8 min) and Cefixime (RT = 3.9 min), at a flow rate of 1.0 mL/min. The chromatogram was recorded at 292nm. System suitability was established by injecting standard solution and results are shown in table no.1.The accuracy of the proposed method was determined by recovery studies. It was confirmed from results that the method is highly accurate (table no.3 and 4). Precision (table no.2) was calculated as interday and intraday variations for both the drugs. Percent relative standard deviations for intraday and interday precision for Azithromycin were 0.30 % and 0.36 % and that for Cefixime were 0.24 % and 0.31 % respectively which are well within the acceptable limit of 2 %. For robustness studies in all deliberately varied conditions, the RSD of contents of Azithromycin and Cefixime were found to be well within the acceptable limit of 2%.

Table 1. Robustness study

Parameters	Changes	Retention Time
Azithromycin		
Flow rate (ml/min)	0.8	3.59
1.2	2.41	
Temperature	40°C	2.890
50°C	2.895	
Cefixime		
Flow rate (ml/min)	0.8	4.75
1.2	3.20	
Temperature	40°C	3.84
50°C	3.86	

Table 2. Precision

S.N.	Sample Weight	Sample Area-1	Sample Area-2	% Assay	% Assay
1	1418.40	482398	473433	100	100
2	1418.40	481010	471886	100	100
3	1418.40	483932	472590	100	100
4	1418.40	480680	473265	100	100
5	1418.40	480544	473320	100	100
6	1418.40	480109	470543	100	100
Average assay				**100**	**100**
STD				**0.30**	**0.24**
%RSD				**0.30**	**0.24**

Table 3. Recovery studies of Azithromycin

S.N.	Spiked Level	Sample Weight (mg)	Sample Area	µg/ml added	µg/ml found	% Recovery
1	50%	709.20	247499	500.00	513.11	103
2	50%	709.20	248653	500.00	515.50	103
3	50%	709.20	246241	500.00	510.50	102
4	50%	709.20	248427	500.00	515.03	103
5	50%	709.20	247861	500.00	513.86	103
6	50%	709.20	241695	500.00	501.07	100
1	100%	1418.40	472870	1000.00	980.34	98
2	100%	1418.40	473206	1000.00	981.03	98
3	100%	1418.40	468669	1000.00	971.63	97
1	150%	2127.60	721023	1500.00	1494.80	100

2	150%	2127.60	722111	1500.00	1497.05	100
3	150%	2127.60	722068	1500.00	1496.96	100
4	150%	2127.60	727747	1500.00	1508.74	101
5	150%	2127.60	726745	1500.00	1506.66	100
6	150%	2127.60	723898	1500.00	1500.76	100

Table 4. Recovery studies of Cefixime

S. N.	Spiked Level	Sample Weight (mg)	Sample Area	µg/ml added	µg/ml found	% Recovery
1	50%	709.20	239014	400.00	403.75	101
2	50%	709.20	237237	400.00	400.75	100
3	50%	709.20	235532	400.00	397.87	99
4	50%	709.20	235248	400.00	397.39	99
5	50%	709.20	237349	400.00	400.94	100
6	50%	709.20	233780	400.00	394.91	99
1	100%	1418.40	462329	800.00	780.98	98
2	100%	1418.40	463545	800.00	783.04	98
3	100%	1418.40	461749	800.00	780.00	98
1	150%	2127.60	706867	1200.00	1194.07	100
2	150%	2127.60	705758	1200.00	1192.19	99
3	150%	2127.60	702577	1200.00	1186.82	99
4	150%	2127.60	700858	1200.00	1183.92	99
5	150%	2127.60	700187	1200.00	1182.78	99
6	150%	2127.60	707884	1200.00	1195.79	100

Fig 1. Azithromycin

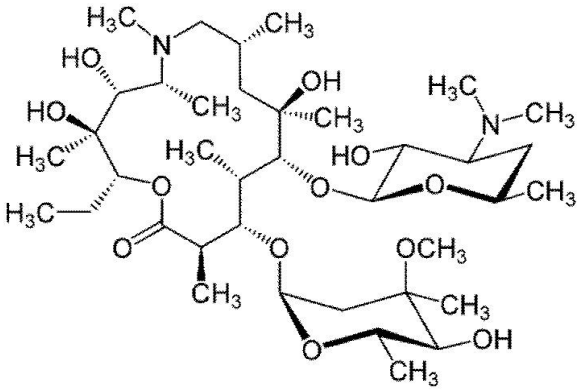


Fig 2. Cefixime

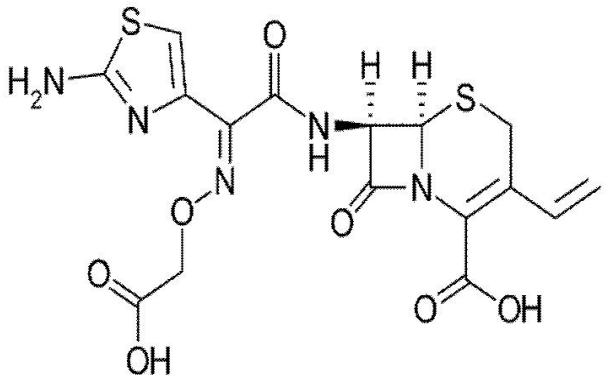


Fig 3. Chromatogram of Test sample

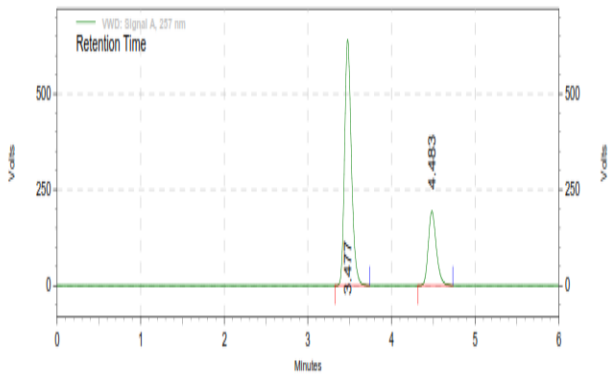


Fig 4. Linearity of Azithromycin

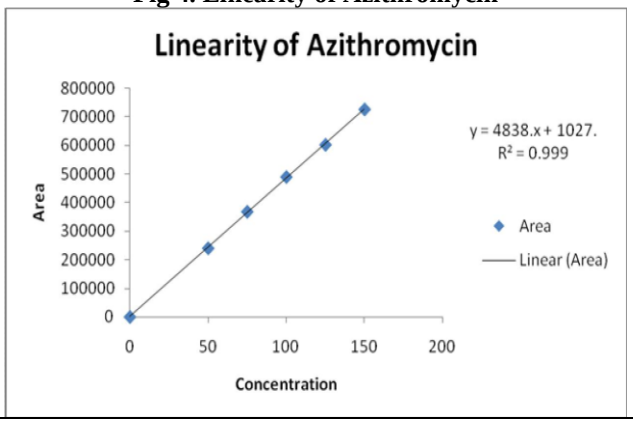
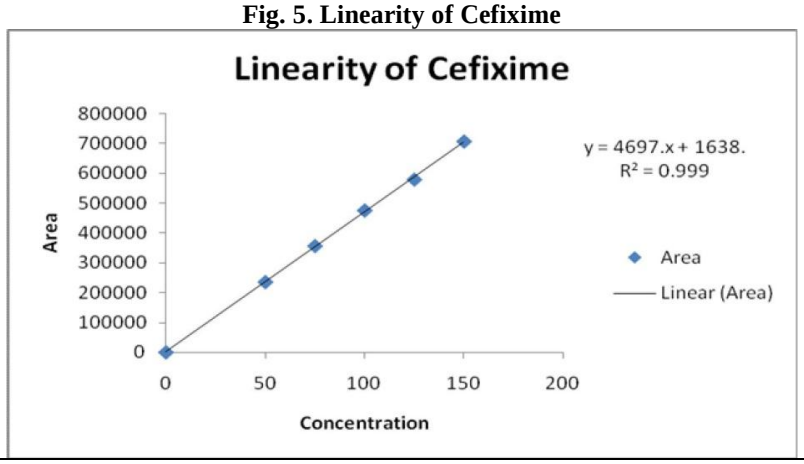


Fig. 5. Linearity of Cefixime



CONCLUSION

The new HPLC method developed and validated for simultaneous estimation of Azithromycin and Cefixime pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, precise, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

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CONFLICT OF INTEREST

No interest

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