ANNEXURE

ANNEXURE I

List of Publications / Paper Presented in Conferences

I) Paper Published


II) Paper Communicated for Publication

1) Prashant D. Ghode and Sunil P. Pawar. 2015. Stability Indicating HPLC Method Development and Validation for the Simultaneous Determination of Cefpodoxime Proxetil and Levofloxacin in its Dosage Forms [Communicated for publication].

IV) Paper Presented in International Conference

ANNEXURE II

STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF LAFUTIDINE & DOMPERIDONE IN ITS DOSAGE FORMS.

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ABSTRACT
A simple, rapid, precise, sensitive and reproducible stability indicating Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for determination of Lafutidine and Domperidone in capsule dosage form was developed. Chromatographic separation was achieved on Zodiac C18 (250mm x 4.6 mm, 5μm) column maintained at ambient temperature eluted with mobile phase at flow rate of 1 ml/min. The mobile phase consists of 0-Phosphoric Acid and Acetonitrile in the ratio 65:35% v/v was used and the determination was carried out at 215nm. The retention time for Lafutidine and Domperidone were 4.901 min and 8.274 min respectively. The linearity was found to be in the range of 3- 9 μg/ml for Lafutidine and 9-27 μg/ml for Domperidone. The analytical method was validated as per ICH guideline for linearity, accuracy, precision, and specificity, limit of detection, limit of quantification, stability etc. and method can be extended to the analysis of Lafutidine and Domperidone in capsule formulations.

KEY WORDS: Lafutidine, Domperidone, Estimation, HPLC, Stability Indicating.

INTRODUCTION
Lafutidine (LAF) as shown in Fig. 1, a second generation H₂ receptor antagonist having multimodal mechanism of action. It has been reported that the gastroprotective effect of LAF is independent of its acid antisecretory activity ¹⁰. LAF not only suppresses gastric acid secretion, but also has cytoprotective properties by the virtue of its property to induce the

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STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF CEPFODOXIME PROXETIL AND OFLOXACIN IN ITS DOSAGE FORMS

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ABSTRACT

A simple, precise, sensitive, reproducible stability indicating Reverse Phase High Performance Liquid Chromatographic method for determination of Cefpodoxime Proxetil and Ofloxacin in tablet dosage form was developed. Chromatographic separation was achieved on Hypersil-keystone RP C₁₈ column maintained at 30°C. Mobile phase consisting of buffer Potassium dihydrogenphosphate: Methanol: Acetonitrile (pH 3.0) in the ratio of 50:30:20 v/v was pumped into the column at a flow rate of 1.2 ml/min. Determination was carried out at 235nm. Two peaks were obtained for Cefpodoxime at 13.1 min and 14.1 min and one peak for Ofloxacin at 5.11 min. The linearity was found to be 4-20 µg/ml and 10-50 µg/ml for Cefpodoxime and Ofloxacin respectively. Method was validated as per ICH guidelines. Cefpodoxime and Ofloxacin were subjected to various stress conditions including acidic, alkaline, oxidation, photolysis, reduction and thermal degradation. The proposed method can be extended to the analysis of Cefpodoxime and Ofloxacin in tablet dosage formulations.

KEYWORD: Cefpodoxime Proxetil, Ofloxacin, HPLC, Stability indicating, validation

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P - 183
Introduction

Cepodoxime Proxil (CFP) is a broad spectrum, orally absorbed third generation cephalosporin antibiotic with chemical name (6R,7R)-7-{[2(Z)-2-Amino-4-thiazolyl] (methoxyimino)- acetyl} amino]-3-\mbox{(methylamino)methyl}-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid 1-{[1-methylthioo} carbonyl]oxyethyl] ester (Fig. 1). It is used in the treatment of influenza, meningitis, gonorrhea, pneumonia, tuberculosis, acute otitis media, pharyngitis. Oflxacin(OFL) is a broad spectrum fluoroquinolones antibacterial with chemical name 6-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazine-1-7-oxo-7H-pyridin-1,2,3-d)1,4-benzoxazine-6-carboxylic acid (Fig. 1). It is used in the treatment of respiratory tract infections, pharyngitis, community-acquired pneumonia, mild to moderate bacterial exacerbation, sexually transmitted diseases, acute and uncomplicated urethral and cervical gonorrhea, urethritis, complicated urinary tract infections, prostatitis. The combination of CFP and OFL has unique dual mode of action, OFL prevents nucleic acid synthesis, while CFP inhibits cell wall synthesis and work synergistically with improved patient compliance. The combined dosage forms of OFLO and CEFPO are available in the market and used as antibacterial drugs. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of OFLO and CEFPO in their combined dosage forms. Literature survey reveals that there are few reported methods to determine CFP\textsuperscript{16} and OFL\textsuperscript{14} alone and in combination with other drugs\textsuperscript{15-16} in dosage forms by spectrometry and HPLC. Few spectrometry\textsuperscript{17,18} and HPLC\textsuperscript{19,20} methods are also available for the simultaneous determination of CEP and OFL but no stability indicating method is available for CEP and OFL in combination. Therefore the purpose of this study is to develop stability indicating method for the simultaneous estimation of CFP and OFL in tablet dosage form.

Materials and Methods

2.1 Chemicals and Reagents

CFP and OFL standards were obtained as a gift samples from Alkem Laboratories, Mumbai. Methanol and acetonitrile of HPLC grade (E. Merck) and potassium dihydrogen phosphate of AR grade (S.D. Fine Chemicals Ltd.) was purchased from local suppliers. Milli Q water was used. All the other reagents were of analytical grade. Marked formulation Zedocof-O (Macleods Pharmaceutical Limited, India) was purchased from local pharmacy.

2.2 HPLC Instruments and analytical conditions

The separation was carried out on HPLC system (Waters) with binary HPLC pump, Waters 2998 PDA detector, Data Module. The chromatographic column used in this study was Hypersil-keystone RP18, (250 mm x 4.6 mm x 5μ). The column was maintained at 30°C. The mobile phase consisting of buffer (20 mM potassium dihydrogen phosphate): methanol: acetonitrile (pH 3.0 adjusted with orthophosphoric acid) was filtered through 0.45μ membrane filter before use, degassed and was pumped from the solvent reservoir in the ratio of 50:30:20[v/v] at a flow rate of 1.2 ml/min. The detection was monitored at 235 nm and the run time was 25min. The volume of injection loop was 10μl. Prior to injection of the drug solution the column was equilibrated for at least 15 min. with the mobile phase flowing through the system. The mobile phase was used as a diluent in the present study.

2.3 Standard and sample preparation

Accurately 200.00 mg of OFL and CEP was weighed and transferred into separate 100 ml volumetric flask containing 30 ml of mobile phase sonicate for 15 min until dissolved. The volume made up to 100 ml with diluent (Stock A of 2000 ppm). Solutions were further diluted with diluent. Twenty tablets of Zedocof-O were accurately weighed and crushed to fine powder. Powdered sample equivalent to 200 mg of CFP (200 mg of OFL) was accurately weighed and transferred into 100 ml
Stability indicating HPLC method development and validation for the simultaneous determination of Azithromycin & Ofloxacin in bulk and its dosage forms

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Abstract

A simple, precise, sensitive and reproducible stability indicating Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for determination of Azithromycin and Ofloxacin in tablet dosage form was developed. Chromatographic separation was achieved on Hypercil-Keystone RP C18 (5 μm, 4.6 mm, 5μm) column maintained at 30 °C eluted with mobile phase at flow rate of 1.2 ml/min. The mobile phase consisted of Buffer (0.02 M potassium dihydrogen phosphate): methanol: acetonitrile in the ratio 65:25:10 v/v at pH maintained at 3.2 with OPA was used and the determination was carried out at 285 nm. The retention time for Azithromycin and Ofloxacin were 9.7 min and 5.01 respectively. The linearity was found in the range of 5–50 μg/ml for Azithromycin and 4–40 μg/ml for Ofloxacin. In stability studies the drugs were well separated from degradation products. The degradation studies were studied in the individual standard drugs, their mixture and formulation which gave the idea about the origin of the degradant products. The analytical method was validated as per ICH guideline for linearity, accuracy, precision, and specificity, limit of detection, limit of quantification, stability in analytical solution etc. and method can be extended to the analysis of Azithromycin and Ofloxacin in tablet formulations.

Keywords: Azithromycin, Ofloxacin, HPLC, Stability Indicating, Validation

1. Introduction

Azithromycin (AZI) is a 15(R, R,S, 8R, 10R, 11R, 12S, 13S, 14S, 15S)-10,11-epoxy-3-O-methyl-6-rib-6-oxa-6-azacycloheptadecan-15-one, one of the semi-synthetic macrolide antibiotic related to Erythromycin. It differs chemically from erythromycin in that a methyl substituted nitrogen atom is incorporated into the lactone ring. Macrolide antibiotics are a bacteriostatic agent that inhibits protein synthesis by binding reversibly to 50S ribosomal subunits of sensitive microorganisms, at or very near the sites that binds chloramphenicol (Figure 1). It is used in the treatment of S. pneumoniae, Community-acquired pneumo- nia due to C. pneumoniae, Pharyngitis or tonsillitis, skin and skin structure infections. Ofloxacin (OFL) is a broad spectrum fluorinated quinolones antibacterial with chemical name 9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4 benzoxazine-6-carboxylic acid (Figure 2). It is used in the treatment of respiratory tract infections, pharyngitis, community-acquired pneumonia, mild to moderate bacterial exacerbation, sexually transmitted diseases, acute and uncomplicated urethral and cervical gonorrhea, urethritis, complicated urinary tract infections, pyelonephritis[1-2].

Figure 1: Structure of Azithromycin

Figure 2: Structure of Ofloxacin

Literature survey reveals that there were few reported methods to determine AZI[3-8] and OFL[9-12] alone in dosage forms by spectrometry and HPLC. Few methods are also available in combination with other drugs[13-22] in dosage forms by spectrometry and HPLC. But no HPLC method is available for the simultaneous estimation of AZI and OFL in its dosage form. Therefore the purpose of this study is to develop stability indicating HPLC method for the simultaneous estimation of AZI and OFL in tablet dosage form.

2.0 Materials and Methods

2.1 Chemicals and Reagents

AZI and OFL standards were obtained as a gift samples from Alkem Laboratories, Mumbai. Methyl and acetonitrile of HPLC grade (E. Merck) and potassium dihydrogen phosphate of AR grade (S.D. Fine Chemicals Ltd.) was purchased from local supplier. Milli Q water was used. All the other reagents were of analytical grade. Marketed formulation Zithromax-O (Alkem Laboratories Limited, Mumbai, India) was purchased from local pharmacy.

2.2 HPLC instruments and analytical conditions

The separation was carried out on HPLC (Waters 2998 with PDA detector), Waters 600 controller (water’s 486 UV visible tunable absorbance detector) were used to develop and validate the method. The chromatographic separation was carried out by using (Hypercil-Keystone RP C18 250 × 4.6 mm, 5μm) column, maintained at 30°C. Data acquisition was done by using Data ace software and Empower 2 software.

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17

Stability Indicating HPLC Method Development and Validation for Simultaneous Estimation of Some Drugs in Bulk and Pharmaceutical Dosage Forms
Stability Indicating HPLC Method Development and Validation for Simultaneous Estimation of Some Drugs in Bulk and Pharmaceutical Dosage Forms

Chapter 10

2.3 Preparation of Mobile phase and Diluent

The mobile phase consisting of buffer (0.02 M potassium dihydrogenophosphate): methanol: acetonitrile (pH 3.2 adjusted with orthophosphoric acid) was filtered through 0.45μm membrane filter before use, degassed and was pumped from the solvent reservoir in the ratio of 65:25:10 v/v at a flow rate of 1.2 ml/min. The detection was monitored at 285 nm and the run time was 25 min. The volume of injection loop was 10μl.

2.4 Preparation of standard and sample (Azithromycin 500 mg + Ofloxacin 400 mg) solutions

Accurately weighed about 500.0 mg of AZI and 400.0 mg of OFL working standards and transferred into separate 100 ml volumetric flasks containing 50 ml of diluent and dissolved by sonication. The volume was made up to 100 ml with diluent (5000 μg/ml and 4000 μg/ml of AZI and OFL respectively- stock A). Solutions were further appropriately diluted to get concentration of 10 μg/ml of AZI and 8 μg/ml of OFL in separate volumetric flask. Ten tablets of Zithromax-250 were accurately weighed and crushed to fine powder. Accurately weighed powder sample (1.231mg) equivalent to 500 mg of AZI (400 mg of OFL) was transferred into 100 ml volumetric flask and dissolved by sonication for 25 min with intermittent shaking. The filtered solution was further diluted to get the concentration 10 μg/ml of AZI (8 μg/ml of OFL).

2.5 Method Development and Validation [23-24]

During mobile phase optimization Buffer: methanol: acetonitrile in the ratio of 65:25:10 at λ285 nm was found to be satisfactory for both drugs. After mobile phase selection, effect of pH and flow rate was studied on resolution of peaks of both the drugs from each other. It was found that pH 3.2 (adjusted with OPA) and flow rate 1.2 ml/min was suitable for both drugs. The method was validated for standard solution for system suitability, selectivity, linearity and range, precision (interday and intraday), accuracy, robustness, ruggedness limit of quantitation (LOQ) and limit of detection (LOD).

For the system suitability study, the standard solutions containing 10 μg/ml of AZI and 8 μg/ml of OFL were prepared and the column efficiency, resolution and peak asymmetry was calculated. The method specificity was assessed by comparing the chromatograms obtained from blank (mobile phase), chromatograms of single drug with the chromatograms of sample. The precision of the method was established by carrying out method precision, system precision, intraday and interday analysis and relative standard deviation (%RSD) was calculated. Method precision and system precision was determined by injecting the sample and standard solutions containing the mixture of 10 μg/ml of AZI and 8 μg/ml of OFL (six replicates) into HPLC system. The intraday precision was studied by performing analysis at regular interval in a day at three different concentration levels (10, 20, 30 μg/ml for AZI and 8, 16, 24 μg/ml for OFL), while interday precision was performed on three different days (day 1, 2, and 3). The accuracy of the method was determined by performing the recovery study by addition of standard drug to the preanalyzed sample at three different levels 80%, 100% and 120% and average percentage recovery was determined. To determine the linearity of the method different concentration levels (5-50 μg/ml for AZI and 4-40 μg/ml for OFL) were prepared from standards stock solutions and each solution was injected into the HPLC system. The peak area of the chromatogram obtained was noted and calibration curve was constructed by plotting response factor against concentration of drugs. Effect of small changes in the HPLC conditions such as change of pH 3.0 & 3.4, change in temperature (25°C-35°C) & change in flow rate (1 & 1.3 ml) was studied to evaluate robustness of the method while ruggedness was studied by performing the assay by the different analyst. The LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve.

2.6 Forced Degradation study

Forced degradation studies were performed to evaluate the stability indicating properties (specificity) of the proposed method. Samples were subjected to stress conditions such as acid hydrolysis, base hydrolysis, heat or thermal, photolytic, oxidation and reduction. Individual drug solutions, standard mixture and sample solution were subjected to the same stress conditions to ensure the effective separation of degradation peaks and main peaks. Standard stock and sample stock solution (stock B) was further diluted to get the concentration 10 μg/ml of AZI and 8 μg/ml of OFL in individual solutions, their mixture and sample solution and same solutions were also kept as control sample. Acid degradation was carried for individual drug solutions, standard mixture and sample solution using 10 ml of 1 N hydrochloric acid; alkali degradation was carried out in 10 ml of 0.1N sodium hydroxide. The stressed solutions were neutralized and then diluted with diluent. Oxidation degradation was performed by adding 10 ml of 30% H2O2 and then diluted with diluent. 10 ml of 10% solution of sodium bisulfite was added in to each flask to carry out the reduction study. For the thermal degradation study solid samples (equivalent to 500 mg of AZI & 400 mg of OFL) and working standards were kept in oven at 60°C for 24 hrs which were further diluted appropriately and chromatographed. Photolytic degradation was carried out in by exposing to UV radiations (1.2 million lux hr). All these solutions were filtered through the 0.45 μm membrane filter before injecting.

3.0 Results and Discussion

3.1 The Finalized Chromatographic Conditions

Based upon system suitability parameters the finalized chromatographic conditions was as follows

<table>
<thead>
<tr>
<th>Column</th>
<th>Hypersil keystone RP C18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave length</td>
<td>285 nm</td>
</tr>
<tr>
<td>Column Temp</td>
<td>30°C</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>16 μl</td>
</tr>
<tr>
<td>Run Time</td>
<td>25 min</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>1.2 ml/min</td>
</tr>
<tr>
<td>Pump Mode</td>
<td>Isocratic</td>
</tr>
<tr>
<td>Retention time</td>
<td>About 5.0 to 5.5 min (For Ofloxacin) About 9.5 to 9.8 min (For Azithromycin)</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>Buffer methanol: acetonitrile (pH 3.2 OPA) Mobile Phase</td>
</tr>
<tr>
<td>Diluent</td>
<td>(0.02 M potassium dihydrogenphosphate) Mobile Phase</td>
</tr>
</tbody>
</table>
Stability Indicating HPLC Method Development and Validation for Simultaneous Estimation of Some Drugs in Bulk and Pharmaceutical Dosage Forms