CHAPTER-4
ORNIDAZOLE, FLOXACIN
AND CEFIXIME
FORMULATIONS
4.1. INTRODUCTION

This chapter covers the method development and validation of RP-HPLC method for the determination of Ornidazole, Ofloxacin and Cefixime in individual and combination pharmaceutical dosage forms. Drug substances therapeutic activity, dose administration and other details, review of literature, materials and methods, results and discussions were described.

4.2. ORNIDAZOLE

Ornidazole (1-4) is a type of concomitant administration of oral anticoagulants may increase the risk of hemorrhage due to diminished hepatic metabolism. Ornidazole belongs to category of concomitant administration of oral anticoagulants may increase the risk of hemorrhage due to diminished hepatic metabolism, drugs. It is a 5-nitroimidazole derivative. Their antimicrobial action is similar to metro-nidazole and is used similarly in the treatment of susceptible protozoal infections and prophylaxis of anaerobic bacterial infections. It has been investigated for use in Crohn's disease after bowel resection. This drug is commonly sold as brand name Dazolic. Ornidazole have antimicrobial activity is due to the reduction of the nitro group to a more reactive amine that attacks microbial DNA, inhibiting further synthesis and causing degradation of existing DNA. Chemical structure of Ornidazole has represented in figure-4.1.

Fig-4.1: Structure of Ornidazole
Chemical details:
Class : Nitroimidazole derivatives anti-protozoals
Chemical Name : Ornidazole
IUPAC name : α-[Chloromethyl]-2-methyl-5-nitroimidazole-1-ethanol-1-
Chloro-3-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol 
Molecular Formula : C\textsubscript{7}H\textsubscript{10}C\textsubscript{1}lN\textsubscript{3}O\textsubscript{3}
Formula Weight : 219.63
CAS No. : 16773-42-5

Dosage and prescribing information for ornidazole
Amoebiasis:
Adults : 500mg twice a day orally for 5 days.
Children : 10-25 mg/kg body weight in two divided doses.

Amoebic dysentery:
Adults : 1.5gms orally once a day for 3 days.
Children : 40 mg/kg body weight, once a day for 3 days.

Giardiasis:
Adults : 1.5 g, once daily for 12 days.
Children : 40mg/kg body weight for 2 days.

Trichomoniasis: 1.5 gm once orally or 500mg twice a day for 5 days.
Bacterial vaginosis: 3 tablets of 500rng each as a single dose or one tablet of 500mg once daily for 5-7 days.

Drug interactions of ornidazole
Concomitant administration of oral anticoagulants may increase the risk of hemorrhage due to diminished hepatic metabolism,

Precautions while prescribing Ornidazole
» Pregnancy & Lactation: To be used only if the potential benefits justify the potential risk to foetus/ neonate.
» In patients with ataxia, vertigo and mental confusion, Ornidazole should be given with caution because the drug has been occasionally reported to aggravate
neurological complications in patients with central and peripheral nervous system disorders.

Incubation of spermatozoa in 2.5 mmol/L Ornidazole for 4h reduced their motility significantly, whereas incubation of epididymal tubules for 8 h in 10 mmol/L Ornidazole was required to alter the velocity parameters of the enclosed spermatozoa upon release, suggesting that extratubular non-metabolized Ornidazole can participate in inhibiting the motility in-vivo. The in vitro toxicity of Ornidazole derivatives depends on the halogen present and on the position of the nitro-group. The putatively inactive (R) and the active (S)-Ornidazole exhibited equivalent depression of sperm motility by direct incubation. This observation, and the differences between the in-vitro and the in vivo efficacies of various Ornidazole analogues, indicates distinct mechanisms of motility inhibition in the two experimental systems.

**Pharmacokinetics**

Ornidazole is readily absorbed from the gastro-intestinal tract and peak plasma concentrations of about 30 ppm have been achieved within 2 hours of a single dose of 1.5 g, falling to about 9 ppm after 24 hours and 2.5 ppm after 48 hours.

The plasma elimination half-life of ornidazole is 12 to 14 hours. Less than 15% is bound to plasma proteins. It is widely distributed in body tissues and fluids, including the cerebrospinal fluid.

Ornidazole is metabolised in the liver and is excreted in the urine, mainly as conjugates and metabolites and to a lesser extent in the faeces; 85% of a single oral dose has been reported to be eliminated within 5 days, 63% in the urine and 22% in the faeces. Biliary excretion may be important in the elimination of ornidazole and its metabolites.

**Indications**

Used in the treatment of severe hepatic and intestinal amoebiasis, giardiasis, trichomoniasis of uro-genital tract and bacterial vaginosis. Also used in the treatment and prophylaxis of susceptible anaerobic infections in dental and
gastrointestinal surgery. Ornidazole is also advocated in the management of *H. pylori* duodenal ulcer in combination with other drugs.

**Warnings and precautions**

Special precautions are required in case of ataxia, vertigo, mental confusion and patients with neurological diseases. There is need for adjustment of dosage and dosage interval in patients with hepatic impairment. Modification of the usual dosage is not necessary in patients of renal failure however; additional post haemodialysis dose may be required in patients undergoing this procedure.

**Usage in pregnancy**

There is no evidence of ornidazole accumulation when used in pregnant women. Therefore, dosage regimen of ornidazole requires no adjustment during pregnancy. However, adequate clinical trials have not been conducted. Ornidazole should be prescribed only if the potential benefit justifies the potential risk to foetus/ neonate.

**Adverse reactions**

The most frequently encountered side effect is dizziness, alone or in combination with other adverse reactions. The other side effects occurring to a lesser extent are nausea, pyrosis, intestinal spasms and metallic taste. Vertigo, fatigue and other discomforts such as loose stools, insomnia, skin rash and headache have also been reported.

**Drug interactions**

So far with Ornidazole, disulfiram like reaction has not been reported on consumption of alcohol.

**Over dosage and treatment**

Over dosage may cause exacerbation of all the pharmacological side effects of Ornidazole. Treat with supportive and symptomatic therapy.
4.3. OFLOXACIN

Ofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class considered to be a second-generation fluoroquinolone. The original brand, Floxin, has been discontinued by the manufacturer in the United States on 18 June 2009, though generic equivalents continue to be available. Ofloxacin chemical structure was shown in figure-4.2.

![Structure of Ofloxacin](image)

**Fig-4.2: Structure of Ofloxacin**

**Chemical details:**
- **Class:** Synthetic chemotherapeutic antibiotic of the fluoroquinolone
- **Chemical name:** Ofloxacin
- **IUPAC 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 or Fluoro Dihydro Methyl (4-methyl 1-piperazinyl) 7-oxo 7H-pyrido 1,4 Benzoxazine 6-Carboxylic acid
- **Mol formula:** C_{18}H_{20}FN_{3}O_{4}
- **Molecular weight:** 361.37
- **CAS NO.:** 82419-36-1

**Medical uses**

Oral and I.V. Floxin is not licensed by the FDA for use in children due to the risk of serious reversible and irreversible injury to the musculoskeletal system. Other fluoroquinolones do have a limited licensed uses in children but are generally not recommended due to safety concerns. Ofloxacin (and its derivatives) has also been associated with a few isolated reports of unexplained pediatric fatalities. Children (those under 18) are also at an increased risk of bone, joint, or tendon injuries.
toxicities. Prescribing ofloxacin in the absence of a proven or strongly suspected bacterial infection or a prophylactic indication is unlikely to provide benefit to the patient and increases the risk of the development of severe adverse drug reactions.

In the adult population ofloxacin is limited to the treatment of proven serious and life threatening bacterial infections such as:

- Acute bacterial exacerbations of chronic bronchitis
- Community-acquired pneumonia
- Uncomplicated skin and skin structure infections
- Nongonococcal urethritis and cervicitis
- Mixed infections of the urethra and cervix
- Acute pelvic inflammatory disease
- Uncomplicated cystitis
- Complicated urinary tract infections
- Prostatitis
- Acute, uncomplicated urethral and cervical gonorrhea.

Ofloxacin has not been shown to be effective in the treatment of syphilis. Floxin is now considered to be contraindicated for the treatment of certain sexually transmitted diseases by some experts due to bacterial resistance.

Ofloxacin administered together with theophylline can lead to elevated blood levels of theophylline. Theophylline is used to open airways in the treatment of asthma. If concurrent use of Ofloxacin and theophylline cannot be avoided, frequent blood tests to monitor theophylline blood levels should be performed. Ofloxacin should be used with caution in patients with central nervous system diseases such as seizures, because rare seizures have been reported in patients receiving this medication. Ofloxacin should be avoided in children and adolescents less than 18 years old, as safe use in these patients have not been established.

Many antibiotics, including Ofloxacin, can alter the normal bacteria in the colon and encourage overgrowth of bacteria responsible for the development of inflammation of the colon (pseudomembranous colitis). Pseudomembranous colitis can cause fever, abdominal pain, diarrhea, and sometimes even shock. Patients
taking Ofloxacin can develop sensitivity of the skin to direct sunlight. Ofloxacin can enhance the action of the anticoagulant warfarin (Coumadin) and increase the risk of bleeding. Both high and low blood sugars have been reported in patients with diabetes taking Ofloxacin together with insulin or other medications used to lower the blood sugar. Careful monitoring of blood sugars is, therefore, recommended when these drugs are concurrently used.

Available forms

Ofloxacin for systemic use is available as tablets (multiple strengths), oral solution (250 mg/mL) and injectable solution (multiple strengths). It is also used as eye drops (trade name Exocin, known as Ocuflox in the United States) and ear drops. Ofloxacin is also used in animals. Its veterinary formulation is sold as Marfloxacin (not to be confused with marbofloxacin, another veterinary-use fluoroquinolone).

Mode of action

Ofloxacin is a broad-spectrum antibiotic that is active against both gram-positive and gram-negative bacteria. It functions by inhibiting DNA gyrase, a type-II topoisomerase and topoisomerase IV, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division.

The fluoroquinolones interfere with DNA replication by inhibiting an enzyme complex called DNA gyrase. This can also affect mammalian cell replication. In particular, some congeners of this drug family display high activity not only against bacterial topoisomerases, but also against eukaryotic topoisomerases and are toxic to cultured mammalian cells and in-vivo tumor models. Although the quinolone is highly toxic to mammalian cells in culture, its mechanism of cytotoxic action is not known. Quinolone induced DNA damage was first reported in 1986.

Recent studies have demonstrated a correlation between mammalian cell cytotoxicity of the quinolones and the induction of micronuclei. As such some fluoroquinolones may cause injury to the chromosome of eukaryotic cells. There is debate as to whether or not this DNA damage is to be considered one of the mechanisms of action concerning the severe and non abating adverse reactions experienced by some patients following fluoroquinolone therapy.
**Contraindications**

As noted above, under licensed use, Ofloxacin is now considered to be contraindicated for the treatment of certain sexually transmitted diseases by some experts due to bacterial resistance.

Due to growing prevalence of antibiotic resistance to the fluoroquinolones in Southeast Asia, the use of Ofloxacin in patients who have been to Southeast Asia is increasingly being contraindicated. Ofloxacin is also considered to be contraindicated within the pediatric population, pregnancy, nursing mothers, patient’s psychiatric illnesses and patients with epilepsy or other seizure disorders.

**Pregnancy**

Research indicates that the fluoroquinolones can rapidly cross the blood-placenta and blood-milk barrier and are extensively distributed into the fetal tissues. Peak concentration in human breast milk is similar to levels attained in plasma. Breast-feeding mothers who take Ofloxacin may expose their infants to severe adverse reactions. Other fluoroquinolones have also been reported as being present in the mother’s milk and are passed on to the nursing child, which may increases the risk of the child suffering from this syndrome as well, even though the child had never been prescribed or taken any of the drugs found within this class.

The data on the safety of the fluoroquinolones in pregnancy contains conflicting reports and is to be considered incomplete due to the lack of adequate studies. But it should be noted that several studies have reported spontaneous abortions following the exposure to the fluoroquinolones during pregnancy, as well as therapeutic/elective abortions due to the perceived, as well as actual, risk of birth defects. However, within one study the authors concluded that the use of quinolones during pregnancy may in some cases be necessary; e.g. drug resistant serious infections, but if safer antibiotics such as penicillin, cephalosporins or erythromycin are an option they should be used instead due to their clearer safety profile.

In regards to Floxin, within a prospective follow-up study of 93 women treated with Ofloxacin during pregnancy, the authors report that there was a higher than expected (11.9%) malformation rate among the infants.
According to the March of Dimes only about 3 to 5 percent of all pregnancies result in children born with birth defects. For this reason the prescribing of Ofloxacin is contraindicated during pregnancy due to the risk of spontaneous abortions and birth defects. Such spontaneous abortions and birth defects have also been found with other drugs within this class, i.e. Ciprofloxacin, Pefloxacin, Norfloxacin and Nalidixic acid. It is generally accepted that the fluoroquinolone class should not be used to treat women who are pregnant due to such risks.

**Pediatric use**

Oral and IV fluoroquinolones including Ofloxacin are not licensed by the FDA for use in children due to the risk of permanent injury to the musculoskeletal system. Within one study it was stated that the pediatric patient has a 3.8% chance of experiencing a serious musculoskeletal adverse event.

However the two most recent pediatric studies involving the use of levofloxacin, the biologically active component of floxin, indicates that the pediatric patient has a greater than 50% chance of experiencing one or more adverse reactions.

**Ofloxacin - clinical pharmacology**

Following oral administration, the bioavailability of Ofloxacin in the tablet formulation is approximately 98%. Maximum serum concentrations are achieved one to two hours after an oral dose. Absorption of Ofloxacin after single or multiple doses of 200 to 400 mg is predictable, and the amount of drug absorbed increases proportionately with the dose. Ofloxacin has biphasic elimination. Following multiple oral doses at steady-state administration, the half-lives are approximately 4 to 5 hours and 20 to 25 hours. However, the longer half-life represents less than 5% of the total AUC. Accumulation at steady-state can be estimated using a half-life of 9 hours. The total clearance and volume of distribution are approximately similar after single or multiple doses. Elimination is mainly by renal excretion. The following are mean peak serum concentrations in healthy 70 to 80 kg male volunteers after single oral doses of 200, 300, or 400 mg of Ofloxacin or after multiple oral doses of 400 mg.
Steady-state concentrations were attained after four oral doses, and the area under the curve (AUC) was approximately 40% higher than the AUC after single doses. Therefore, after multiple-dose administration of 200 mg and 300 mg doses, peak serum levels of 2.2ppm and 3.6ppm, respectively, are predicted at steady-state.

4.4. CEFIXIME

Cefixime\(^{46-47}\) is used to treat a wide variety of bacterial infections. This medication is known as a cephalosporin antibiotic. It works by stopping the growth of bacteria. This antibiotic treats only bacterial infections. It will not work for viral infections (e.g., common cold, flu). Unnecessary use or overuse of any antibiotic can lead to its decreased effectiveness.

Take this medication by mouth with or without food, usually once a day. The dosage is based on your medical condition and response to therapy. Antibiotics work best when the amount of medicine in your body is kept at a constant level. Therefore, take this drug at evenly spaced intervals. Continue to use this medication until the full prescribed amount is finished, even if symptoms disappear after a few days. Stopping the medication too early may allow bacteria to continue to grow, which may result in a relapse of the infection.

Cefixime is an oral third generation cephalosporin antibiotic. Cefixime is a cephalosporin antibiotic used to treat infections caused by bacteria such as pneumonia; bronchitis; gonorrhea; and ear, lung, throat, and urinary tract infections. It was sold under the trade name Suprax in the USA until 2003 when it was taken off the market by drug manufacturer Wyeth after its patent expired. The oral suspension form of "Suprax" was re-launched by Lupin in the USA. The structure of Cefixime has represented in figure-4.3.

\[\text{OH}\]

Fig-4.3: Structure of Cefixime

165
Chemical details:

Class : Third generation cephalosporin antibiotic.
Chemical name : Cefixime
IUPAC name : (6R, 7R)-7-[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
Molecular formula : $C_{16}H_{15}N_{5}O_{7}S_{2}$
Formula weight : 453.45
CAS NO. : 79350-37-1

Cefixime is a cephalosporin antibiotic used to treat infections caused by bacteria such as pneumonia; bronchitis; gonorrhea and ear, lung, throat and urinary tract infections. Antibiotics will not work for colds, flu, or other viral infections. This medication is sometimes prescribed for other uses; ask your doctor or pharmacist for more information.

Cefixime comes as a tablet and liquid to take by mouth. It is usually taken once a day or every 12 hours (twice a day) for 5-14 days. Gonorrhea may be treated in 1-10 days. Follow the directions on your prescription label carefully.

Drug class and mechanism:

Cefixime is a semi-synthetic (partially man-made), oral antibiotic in the cephalosporin family of antibiotics. The cephalosporin family includes cephalexin (keflex), cefaclor (ceclor), cefuroxime (zinacef), cefpodoxime (vantin), cefprozil (cefzil), and many injectable forms. Like other cephalosporins, cefixime stops bacteria from multiplying by preventing bacteria from forming the walls that surround them. The walls are necessary to protect bacteria from their environment and to keep the contents of the bacterial cell together; bacteria cannot survive without a cell wall. Cefixime is active against a very wide spectrum of bacteria such as Staphylococcus aureus, streptococcus pneumoniae, streptococcus pyogenes (the cause of strep throat), hemophilus influenzae, moraxella catarrhalis, E. coli, klebsiella, proteus mirabilis, salmonella, shigella, and neisseria gonorrhoeae.
Prescribed for:

Cefixime is effective for infections of the middle ear (otitis media), tonsillitis, throat infections (pharyngitis), laryngitis, bronchitis and pneumonia caused by susceptible bacteria. It is also used for treating urinary tract infections and gonorrhea as well as acute bacterial bronchitis in patients with chronic obstructive pulmonary disease (COPD).

Drug interactions:

Probenecid (benemid) may increase the blood concentration of cefixime by decreasing removal of cefixime by the kidney. This interaction sometimes is used to enhance the effect of cephalosporins. Combining cefixime with aminoglycosides [for example, tobramycin (tobradex) produces additive bacterial killing effects but also may increase the risk of harmful effects to the kidney.

Exenatide (byetta) may delay or reduce the absorption of cephalosporins. Cephalosporins should be administered one hour before exenatide. Cefixime may cause a false positive urine ketone test.

Pregnancy:

Safety in pregnancy has not been established for cefixime. There are no adequate studies in pregnant women; however, studies in animals suggest no important effects on the fetus.

Nursing mothers:

Safety in nursing mothers has not been established. It is not known if cefixime is excreted in breast milk.

Side effects:

Cefixime is generally well tolerated and side effects usually are transient. Reported side effects include diarrhea, nausea, abdominal pain, vomiting, skin rash, fever, joint pain and arthritis, abnormal liver tests, vaginitis, itching, headaches and dizziness.

Cefixime should be avoided by patients with a known allergy to cephalosporin type antibiotics. Since cefixime is chemically related to penicillin, an
occasional patient can have an allergic reaction (sometimes even life-threatening anaphylaxis) to both medications.

Like most antibiotics cefixime may cause a condition called pseudomembranous colitis, a potentially serious bacterial infection of the colon caused by a bacterium called clostridium difficile (C. difficile colitis). Patients who develop pseudomembranous colitis as a result of antibiotic treatment can experience diarrhea, abdominal pain, fever and sometimes even shock.

**Pharmacokinetics**

Cefixime binds to one or more of the penicillin-binding proteins (PBPs) which inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell wall, thus inhibiting biosynthesis and arresting cell wall assembly resulting in bacterial cell death.

**Absorption**

Only 40-50% is absorbed from the GI tract (oral); rate may be decreased if taken with food. Greater absorption observed in oral suspension than tablets.

**Excretion**

20% of an oral dose excreted via urine unchanged; 60% nonrenal elimination; some is excreted via the faeces from the bile. Substantially removed by dialysis.

**Dosage**

**Adult:** 200-400 mg/day as a single dose or in 2 divided doses.

**Child:** 8 mg/kg/day as a single dose or in 2 divided doses; < 6 months are not recommended.

Treatment should be continued for 48 hours after disappearance of symptoms. Renal impairment Dose reduction is necessary. CrCl ml/min Dosagerecommendation<20 to Max:200mgdaily.

**Oral**

Uncomplicated gonorrhoea Adult: 400 mg as a single dose.

Renal impairment: Dosereductionisnecessary. CrCl [mL/m] Dosage Recommendation <20 to Max: 200 mg daily.
Cefixime is a third-generation cephalosporin that is used for the treatment of infections caused by various pathogens, especially gram-negative bacteria. It is a generally well-tolerated and safe antibiotic. Its common adverse effects are gastrointestinal disturbances (such as diarrhea and stool changes), headache, dizziness, fatigue, muscle aches, and strange taste in the mouth. Hepatotoxicity associated with cefixime has not been noted previously in the medical literature. In this report, we present a case of cefixime-induced hepatotoxicity. A 50-year-old female was admitted with loss of appetite and abdominal pain. She had been treated for an upper respiratory infection with oral cefixime for 7 days by her physician. Her complaints emerged 5 days following the treatment. In her medical history, she denied alcohol, herbal products and other drug use. She had no recent travel history. Physical examination was normal except for tenderness in the right upper quadrant. Laboratory tests results were as follows: alanine aminotransferase (ALT): 156 U/L (normal <40 U/L), aspartate aminotransferase (AST): 56 U/L (normal <40 U/L), and gamma glutamyl transpeptidase (GGT): 281 U/L (normal <55 U/L). Alkaline phosphatase (ALP), bilirubin, albumin and prothrombin time were within the normal range. Autoimmune hepatitis markers, thyroid tests, ferritin and ceruloplasmin were also normal. Viral markers for A, B, and C were negative. Ultrasound revealed no evidence of hepatobiliary-pancreatic pathology. These findings were attributed to hepatocellular type liver damage associated with cefixime use. After a two-week withdrawal of the drug, serum transaminase levels returned to normal range. The patient did not undergo percutaneous liver biopsy as the serum enzyme levels returned to normal range, and she did not consent to the biopsy procedure. The diagnosis was established as cefixime-induced hepatotoxicity based on the absence of drug use history and other causes of hepatitis. To our knowledge, this case is the first presentation of hepatotoxicity associated with cefixime. In conclusion, even though cefixime is generally known to be a well-tolerated and safe antibiotic, physicians should be aware of the risk of hepatotoxicity associated with cefixime use.
4.4. REVIEW OF LITERATURE

TsingHua (48) (2003), a reversed HPLC method was established for the determination of ornidazole in human plasma. Its pharmacokinetic investigation was studied in healthy volunteers. Methods The drug was extracted from plasma with methanol and isopropanol (50:50), and then detected by UV detector at 316 nm with a C18 5 μ, 250 nm × 4.6 mm column. The mixture of methanol and 0.4% glacial acetic acid (50:50) was used as the mobile phase. The flow rate was 0.8 mL/min.

RESULTS: The calibration curve was linear over the range of 2.0 ~ 20.0 ppm and the measurable limit of detection was 0.2 ppm. The average recoveries of ornidazole at the concentrations of 2.0, 10.0, 20.0 ppm were 100.36%, 98.21% and 97.42%, respectively. The RSDs of the within-day and between-day variations were less than 7% and 6%, respectively. The results showed that the concentration time curves of the two preparations were fitted to a two compartment model with a lag time.

CONCLUSION: This HPLC method is simple, sensitive and accurate. It is suitable for routine determination of ornidazole levels in human plasma and for its pharmacokinetic study.

Natarajan SunderS (49) (2005) a single, simple, fast, precise, accurate and rugged stability indicating reversed phase HPLC method has been developed and validated for the simultaneous estimation of Ofloxacin and Ornidazole in tablets. The stability indicating capability of the method was proven by subjecting the drugs to stress conditions of alkaline and acidic hydrolysis, oxidation, photolysis and thermal degradation and resolution of the degradation products formed therein. The separation was obtained using a mobile phase of 80:20 ratio of pH 3.0 buffer and acetonitrile on a ODS (octa decyl silane) column (4.6 mm × 250 mm, 5 μ) with UV detection at 300 nm at 1 ml per minute flow rate. For stress studies, a diode array detector was used. The elution order was Ofloxacin followed by Ornidazole. The linear dynamic range was 40-140 ppm and 100-350 ppm for Ofloxacin and Ornidazole, respectively. Percentage recoveries for Ofloxacin and Ornidazole were 100% and 99.81%. The method validation showed excellent results for precision,
accuracy, linearity, specificity, limit of detection, limit of quantitation and robustness.

Siva Kumar R (50) et al., (2009) have developed a reverse Phase HPLC method for the determination of nitazoxanide and ofloxacin in bulk and tablet formulations. The determination was carried out by using Phenomenex C18 column with 0.24% sodium lauryl sulphate: acetonitrile: acetic acid (pH-4.0) (58:40:02) as the mobile phase. The flow rate was 1.5 mL/ min. and the eluent was monitored at 295 nm. The retention time of nitazoxanide and ofloxacin were 2.2 and 5.4 respectively. Linearity for the nitazoxanide and ofloxacin were found in the range of 400-600 ppm and 160 - 240 ppm respectively. The method was reproducible, with good resolution between nitazoxanide and ofloxacin and can be use for routine analysis.

Nanda RK (51) (2009) two accurate, precise, rapid and economical methods were developed for the estimation of Cefixime and Ornidazole in tablet dosage form. First method is based on the simultaneous equations and wavelengths selected for analysis were 290.0 nm (λmax of Cefixime) and 312.0 nm (λmax of Ornidazole) respectively, in methanol. Second method is Qanalysis method based on absorbance ratio at two selected wavelengths 303.0 nm (iso-absorptive point) and 312.0 nm (λmax of Ornidazole). The linearity was obtained in the concentration range of 10-50 for both Cefixime and Ornidazole. The proposed procedures were successfully applied for the simultaneous determination of both drugs in commercial tablet preparation. The results of the analysis have been validated statistically and by recovery studies.

Pande V.V (52) (2009) describes a new rapid, easy Isocratic reversed phase HPLC method for the separation and estimation of intermediate as 2-methyl 5-nitro imidazole and Ornidazole. The primary purpose of this study is to compile HPLC data on the determination of these products, even though they belong to one group, the compilation of such HPLC data being useful as reference guide. It was also carried out to study the feasibility of determination of any individual / combination of three intermediate using the four as the internal standards.
Dhandapani et al. (2010) developed and subsequently validated a simple reversed phase liquid chromatographic method for simultaneous determination of Ofloxacin and Ornidazole in combination. The separation was carried out using a mobile phase consisting of 2mM phosphate buffer and Acetonitrile with pH 3.5 adjusted with ortho phosphoric acid in the ratio of 70:30%v/v. The column used was Phenomenex C18, (250 mm x 4.6 mm i.d, 5μ) with flow rate of 1 mL/min using PDA detection at 293 nm. The described method was linear over a concentration range of 5-50 ppm and 12.5-125 ppm for the assay of Ofloxacin and Ornidazole respectively. Gatifloxacin (50 ppm) was used as internal standard. The retention times of Ofloxacin, Ornidazole and Gatifloxacin were found to be 2.1, 2.5 and 5.5min respectively. Results of analysis were validated statistically and by recovery studies. The limit of detection (LOD) and the limit of quantification (LOQ) for Ofloxacin and Ornidazole were found to be 5 and 10 ppm 10 and 25 ppm respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Ofloxacin and Ornidazole bulk drug and in its pharmaceutical dosage form.

Manisha Puranik (2010) developed and validated simple, rapid and accurate chromatographic methods for simultaneous determination of ofloxacin and ornidazole in solid dosage form. The first method was based on reversed phase high performance liquid chromatography, on Intersil C18 column (250 mm, 4.6 i.d.), using acetonitrile: methanol: 0.025M phosphate buffer, pH 3.0 (30:10:60 % v/v/v) as the mobile phase, at a flow rate of 1 mL/min at ambient temperature. Quantification was achieved with UV detection at 318 nm over a concentration range of 2-40 ppm for ofloxacin and 5-100 ppm for ornidazole. The mean retention time of ofloxacin and ornidazole was found to be 4.04 min and 5.83 min, 6.77 min (isomers), respectively. The amount of Ofloxacin and Ornidazole estimated as percentage of label claimed was found to be 100.23 and 99.61%, with mean percent recoveries 100.20 and 100.93%, respectively. The second method was based on TLC separation of these drugs using silica gel 60F254 aluminium sheets and dichloromethane: methanol: 25% ammonia solution (9.5:1:3 drops v/v) as mobile phase. Detection
was carried out at 318 nm over the concentration range of 20-100 ng/spot for Ofloxacin and 50-250 ng/spot for Ornidazole. The mean Rf value of Ofloxacin and Ornidazole was found to be 0.16 and 0.56, 0.78 (isomers), respectively. The amount of Ofloxacin and Ornidazole estimated as percentage of label claimed was found to be 100.23 and 99.61% with mean percent recoveries 100.47 and 99.32%, respectively. Both these methods were found to be simple, precise, accurate, selective and rapid and could be successfully applied for the determination of pure laboratory prepared mixtures and tablets.

Sudhakar et al. (2010) a selective and sensitive reverse phase high performance liquid chromatography (RP-HPLC) has been first developed for the separation and quantification of cefixime trihydrate and ornidazole in tablet dosage form and validated. The determination was carried out using Phenomenex C18 column (25 cm × 4.6 mm id) as a stationary phase and mobile phase comprised of acetonitrile: 40mM KH2PO4 in proportion of 40:60(v/v) with pH adjusted to 6±0.5 by using triethylamine. The flow rate was 1.0mL/min and the eluent was monitored at 310nm. The retention time of cefixime trihydrate and ornidazole were 2.75 ±0.028 min and 6.67±0.018 min respectively. The method was validated in terms of linearity, precision, accuracy, ruggedness and specificity, limit of detection and limit of quantification. The Coefficient of correlation and percentage recoveries of cefixime trihydrate and ornidazole were 0.9986 and 100.01 % and 0.9994 and 99.98% respectively. The developed HPLC method was extended for dissolution studies. The dissolution testing was performed at 50 rpm and 100 rpm in 0.1N HCl as dissolution medium by paddle method.

Mônica Felts de La Roca Soares (2010) has developed and validated a new dissolution test for ornidazole coated tablets. The dissolution conditions were determined after testing Sink conditions, dissolution medium, apparatus, stirring speed, 24 h stability and medium filtration influence. The best conditions were paddle at a stirring speed of 75 rpm and 900 mL of 0.1 M HCl. A new HPLC quantification method was developed and validated. The dissolution test and quantification method showed to be adequate for their purposes and could be
applied for quality control of ornidazole coated tablets, since there is no official monograph.

4.5. OBJECTIVE

All reported methods are chemical and instrumental methods for the determination of ornidazole, ofloxacin and cefixime in individually and two combinations and no single method reported for the determination of three ingredients simultaneously. The present research work objective is to develop and single method for three ingredients determination by using RP-HPLC.

4.6. MATERIALS AND METHODS

Reagents:
Water : Millipore
Methanol : AR grade
Orthophosphoric acid : AR grade

Mobile phase:
Mobile phase prepared with high pure chemicals. Mixed the methanol: water in the ratio of 70:30% (v/v), adjusted the pH to 5.2 with diluted orthophosphoric acid and mixed well. Filtered and degased with 0.45μ filter.

Diluent: Mobile phase.

Chromatographic conditions:
Column : Kromasil C18, 250mm x 4.6mm, 5μ or Equivalent
Flow : 1.0mL / minute
Wavelength : 220nm
Column Temperature : 25°C
Injection Volume : 20μL
Run Time : 12minutes
Retention time of actives : Cefixime-3.7min, Ornidazole-6.2min and Ofloxacin-8.7min.
**Standard solution:**

Weighed accurately and transferred 100.0mg of each Ornidazole, Ofloxacin and Cefixime standards into a 100mL volumetric flask, added 70mL of diluent, sonicated for 20 minutes and makeup to 100mL with diluent. Transferred 5mL of the above solution into a 50mL volumetric flask and makeup to 50mL with diluent.

**Preparation of test solution:**

Weighed accurately and transferred equivalent to 100mg of each active ingredient (Ornidazole, Ofloxacin and Cefixime) into a 100mL volumetric flask, added about 70mL of diluent, sonicated for 30 minutes and makeup to 100mL with diluent. Transferred 5mL of the above solution into a 50mL volumetric flask and makeup to 50mL with diluent.

**System suitability solution:**

3. The tailing factor of each active ingredient peak in standard solution is not more than 2.0.

4. The % of RSD of each active ingredient peak area in five replicate standard injections is not more than 2.0%.

**Calculation:**

\[
\text{% of Active ingredient} = \frac{A_T \times \text{Std wt} \times \text{Avg wt of tablet/capsules} \times P}{A_S \times \text{sample wt} \times \text{Label claim}}
\]

Where, \( A_T \) and \( A_S \) are the areas of test and standard solutions respectively. 

\( P \) is the potency of standard.

**Acceptance criteria:** The limit of assay is in between the 98% - 102%.

Detailed method development was discussed in results and discussion.

**4.7. RESULTS AND DISCUSSION**

A single RP-HPLC method developed and validated for the quantification of Ornidazole, Ofloxacin and Cefixime in bulk and formulations. The details of method optimization and method validation were reported below.
Selection of detector wave length:

Wave length selection has performed with the preparation of standard solution by using high pure standards. Standard solutions were scanned from 200nm to 400nm with UV spectrophotometer. Results were all active ingredients have maximum absorbance at 210nm, 254nm and above 300nm. Since all the three compounds maximum absorbance coinsiding at 220nm so UV absorbance was measure for diluent, standard and test sample at 220nm.

Diluent selection:

Based on the literature survey active ingredients solubility is,

**Ornidazole:** Very soluble in Ethanol, chloroform and acetic acid, slightly soluble in water and almost insoluble in petroleum ether.

**Ofloxacin:** Slightly soluble in water, soluble in glacial acetic acid, slightly soluble or soluble in dichloromethane, slightly soluble in methanol.

**Cefixime:** Slightly soluble in alcohol, acetone and glycerin, very slightly soluble in 70% of sorbitol and octanol. Practically insoluble in ether, ethyl acetate, hexane and water.

Based on the above solubility data checked the solubility with different solvents individually and mixed composition. Finally the selected the stable diluent for three ingredients is mobile phase.

Selection of HPLC column:

Initial HPLC method development trial was with phosphate buffer and methanol, reverse phase C18 (250x4.6mm, 5μ) column but with this method, Ornidazole, Ofloxacin and Cefixime peak shapes were poor. Finally, in optimized chromatographic conditions Kromasil C18 column was used and this column gave reproducible results.

Selection of run time and flow rate:

In optimized conditions, the retention time of Ornidazole is 6.2min, Ofloxacin is 8.7min and Cefixime is 3.76min, respectively. Hence the run time was finalized to 12minutes and with a mobile phase flow rate of 1.0mL/ min.

Selection of standard and sample solution concentration:
Test samples obtained from reputed pharmaceutical industry. The standard concentration is based on the response of active ingredients. Standard and sample concentrations were 100ppm for each active ingredient (Ornidazole, Ofloxacin and Cefixime).

Selection of other chromatographic parameters:

UV Spectrophotometry and HPLC are most commonly used for the analysis of dissolution samples. UV is the more preferable choice when compared to HPLC because of the time required for the analysis of the sample. In cases when the interference is higher than 2.0%, it is recommended to choose another wavelength. Regarding interference from excipients, sensitivity issues, automated system and regulatory agencies, an HPLC method for the determination of Ornidazole, Ofloxacin and Cefixime in tablets assay and after dissolution also recommended, especially if it has a short retention time.

The starting point for the development of the assay of three ingredients were the chromatographic conditions of a 5μ C8 column (250 X 4.6 mm) using mobile phase consisting of a mixture of methanol: acetonitrile: water: 0.1% ortho phosphoric acid (35:5:5:5% v/v/v/v), adjusted the pH to 3.0 with diluted triethylamine. The flow rate was maintained at 1.0 mL/min. The detection of the constituents was done using UV detector at 230nm.

**Trial -1: Chromatographic conditions**

<table>
<thead>
<tr>
<th>Column</th>
<th>Merck make Lichrospher C8 250×4.0mm, 5μ.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detector</td>
<td>220nm</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.2mL/min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>50μL</td>
</tr>
<tr>
<td>Run time</td>
<td>20min</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>Buffer (pH: 3.5 phosphate): acetonitrile: methanol (40:40:20)</td>
</tr>
</tbody>
</table>

Std/spl concentration : 70ppm for each ingredient.

**Conclusion:** Three ingredients peak shape was poor and the resolution also not acceptable.
**Trial -1: Chromatographic conditions**

<table>
<thead>
<tr>
<th><strong>Column</strong></th>
<th>Hypersil C$_{18}$, 150×4.6mm, 5µ.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Detector</strong></td>
<td>220nm</td>
</tr>
<tr>
<td><strong>Flow rate</strong></td>
<td>0.8mL/min</td>
</tr>
<tr>
<td><strong>Injection volume</strong></td>
<td>10µL</td>
</tr>
<tr>
<td><strong>Run time</strong></td>
<td>250min</td>
</tr>
<tr>
<td><strong>Mobile phase</strong></td>
<td>Buffer (1.0gm of ammonium acetate in 1000mL of water): acetonitrile (60:40v/v)</td>
</tr>
</tbody>
</table>

**Conclusion:** Ofloxacin was eluted with broad peak with late elution.

Final method was evaluated with the system suitability parameters. Figures-4.4 to 4.6 represents the individual injections of three actives.

![Fig-4.4: Cefixime individual chromatogram](image-url)
System suitability

Blank and standard solutions were injected to demonstrate the system suitability. Resolution, tailing, theoretical plates and peak purity aspects are within the specification. Chromatograms of five replicate standard solutions are given in Figures 4.7 and 4.11.
Fig-4.7: Standard solution-1\textsuperscript{st} chromatogram

Fig-4.8: Standard solution-2\textsuperscript{nd} chromatogram
Fig-4.9: Standard solution-3rd chromatogram

Fig-4.10: Standard solution-4th chromatogram
Specificity

To examine the applicability of the method, interferences of blank and placebo were checked. The peak purity (purity angle should be less than purity threshold) of all the active ingredients in standard solution indicate that the peaks are homogeneous and all compounds are well separated from each other which shows specificity of the method.

In order to confirm the specificity, stress studies were performed with different conditions including acid hydrolysis, base hydrolysis, H2O2 oxidation, dry heat, heat/humidity, and light exposure on both solution and solid state of the sample. Sufficient degradation was allowed for an accurate evaluation of the method to quantitate the analytes of interest in the presence of relevant degradation products. The stressed samples were then analyzed on a UPLC instrument equipped with a photodiode array detector. Purity threshold is more than purity angle is an indication of the absence of co-elution of peaks.

Linearity and range

The linearity of the method was determined by using different concentration of mixture of three active ingredients of the specification limit were prepared and
analysed. The peak response verses concentration of data was treated by linear regression analysis for each ingredient was performed. (Acceptance criterion for linearity is correlation coefficient should not be less than 0.999). Linerity chromatograms are shown in Figures-4.12 to 4.16 relative response factors were established based upon the slope of calibration plots.

**Fig-4.12: Linearity level-1st chromatogram**
Fig-4.13: Linearity level-2\textsuperscript{nd} chromatogram

Fig-4.14: Linearity level-3\textsuperscript{rd} chromatogram

Fig-4.15: Linearity level-4\textsuperscript{th} chromatogram
Fig-4.16: Linearity level-5th chromatogram

Linearity results were tabulated in tables- 4.1 to 4.3. Linearity graphs were plotted between area and concentration and represented in figures-4.17 to 4.19.

Table-4.1: Ornidazole linearity results

<table>
<thead>
<tr>
<th>Level</th>
<th>Concentration of Ornidazole in ppm</th>
<th>peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level - 1</td>
<td>60</td>
<td>322266</td>
</tr>
<tr>
<td>Level - 2</td>
<td>80</td>
<td>416017</td>
</tr>
<tr>
<td>Level - 3</td>
<td>100</td>
<td>502381</td>
</tr>
<tr>
<td>Level - 4</td>
<td>120</td>
<td>589746</td>
</tr>
<tr>
<td>Level - 5</td>
<td>140</td>
<td>682930</td>
</tr>
</tbody>
</table>

Range:60ppm to 140ppm

slope 4475
intercept 55139
correlation coefficient 0.9998
Table-4.2: Ofloxacin linearity results

<table>
<thead>
<tr>
<th>Level</th>
<th>Concentration of Ofloxacin in ppm</th>
<th>peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level - 1</td>
<td>60</td>
<td>258919</td>
</tr>
<tr>
<td>Level - 2</td>
<td>80</td>
<td>335326</td>
</tr>
<tr>
<td>Level - 3</td>
<td>100</td>
<td>408544</td>
</tr>
<tr>
<td>Level - 4</td>
<td>120</td>
<td>476379</td>
</tr>
<tr>
<td>Level - 5</td>
<td>140</td>
<td>564370</td>
</tr>
<tr>
<td>Range: 60ppm to 140ppm</td>
<td>slope</td>
<td>3759</td>
</tr>
<tr>
<td></td>
<td>intercept</td>
<td>32730</td>
</tr>
<tr>
<td></td>
<td>correlation coefficient</td>
<td>0.9992</td>
</tr>
</tbody>
</table>

Table-4.3: Cefixime linearity results

<table>
<thead>
<tr>
<th>Level</th>
<th>Concentration of Cefixime in ppm</th>
<th>peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level - 1</td>
<td>60</td>
<td>234962</td>
</tr>
<tr>
<td>Level - 2</td>
<td>80</td>
<td>304510</td>
</tr>
<tr>
<td>Level - 3</td>
<td>100</td>
<td>364321</td>
</tr>
<tr>
<td>Level - 4</td>
<td>120</td>
<td>441791</td>
</tr>
<tr>
<td>Level - 5</td>
<td>140</td>
<td>501856</td>
</tr>
<tr>
<td>Range: 60ppm to 140ppm</td>
<td>slope</td>
<td>3355</td>
</tr>
<tr>
<td></td>
<td>intercept</td>
<td>33953.5</td>
</tr>
<tr>
<td></td>
<td>correlation coefficient</td>
<td>0.9993</td>
</tr>
</tbody>
</table>
Fig-4.17: Ornidazole linearity graph

Fig-4.18: Ofloxacin linearity graph
Accuracy

Accuracy of the method was evaluated with spiking method. Standard was added to the placebo and analyzed with the optimized chromatographic conditions. Each active ingredient standard stock solution added to the placebo with known quantities from 100% and 150% (50%, 100% and 150%) of the specification level. Results were satisfactory and tabulated in the below table-4.4.

Table-4.4: Accuracy of the method

<table>
<thead>
<tr>
<th>Level</th>
<th>% Recovery</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 % Level</td>
<td>100 % Level</td>
</tr>
<tr>
<td>Ornidazole</td>
<td>95.40</td>
<td>93.60</td>
</tr>
<tr>
<td></td>
<td>105.60</td>
<td>99.30</td>
</tr>
<tr>
<td></td>
<td>108.30</td>
<td>99.40</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>109.70</td>
<td>101.20</td>
</tr>
<tr>
<td></td>
<td>101.20</td>
<td>102.10</td>
</tr>
<tr>
<td></td>
<td>99.50</td>
<td>99.60</td>
</tr>
<tr>
<td>Cefixime</td>
<td>100.20</td>
<td>100.80</td>
</tr>
<tr>
<td></td>
<td>101.50</td>
<td>99.80</td>
</tr>
<tr>
<td></td>
<td>98.90</td>
<td>101.20</td>
</tr>
</tbody>
</table>

Fig-4.19: Cefixime linearity graph
**Precision**

To demonstrate the system precision, six replicate injections of standard solution were given in the HPLC system. The % RSD of all the components was found to be within the specified limit. Chromatograms of six different preparations were represented in figure-4. Data of the system precision are given in the Tables-4.5 to 4.7.

**Table-4.5: Precision Results for cefexime (Analyst-1)**

<table>
<thead>
<tr>
<th>Conc. (in ppm)</th>
<th>Injection No.</th>
<th>Peak areas</th>
<th>RSD (Acceptance criteria ≤ 2.0%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1</td>
<td>347905</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>344551</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>349112</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>346072</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>355023</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>352521</td>
<td></td>
</tr>
</tbody>
</table>

**Table-4.6: Precision results for Ornidazole (Analyst-1)**

<table>
<thead>
<tr>
<th>Conc. (in ppm)</th>
<th>Injection No.</th>
<th>Peak areas</th>
<th>RSD (Acceptance criteria ≤ 2.0%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1</td>
<td>496841</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>502166</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>492131</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>505951</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>487377</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>493059</td>
<td></td>
</tr>
</tbody>
</table>
**Table-4.7:** Precision results for Ofaxacin (Analyst-1)

<table>
<thead>
<tr>
<th>Conc. (in ppm)</th>
<th>Injection No.</th>
<th>Peak areas</th>
<th>RSD (Acceptance criteria ≤ 2.0%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1</td>
<td>414905</td>
<td>0.838</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>416504</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>416349</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>520915</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>410940</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>412587</td>
<td></td>
</tr>
</tbody>
</table>

**Intermediate precision:**

The Intermediate precision was demonstrated by analyzing the six preparations with two different analysts; two different instruments and different lot of the same columns on different days were verified. Results in Tables-4.8 to 4.10 indicate the intermediate method precision of the finished product. The overall % RSD was found to be within the specification.

**Table-4.8:** Precision Results for cefexime (Analyst-2)

<table>
<thead>
<tr>
<th>Conc. (in ppm)</th>
<th>Injection No.</th>
<th>Peak areas</th>
<th>RSD (Acceptance criteria ≤ 2.0%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1</td>
<td>347905</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>344551</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>349112</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>346072</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>355023</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>352521</td>
<td></td>
</tr>
</tbody>
</table>
Table-4.9: Precision Results for Ornidazole (Analyst-2)

<table>
<thead>
<tr>
<th>Conc. (in ppm)</th>
<th>Injection No.</th>
<th>Peak areas</th>
<th>RSD (Acceptance criteria ≤ 2.0%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1</td>
<td>496841</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>502166</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>492131</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>505951</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>487377</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>493059</td>
<td></td>
</tr>
</tbody>
</table>

Table-4.10: Precision results for Ofaxacin (Analyst-2)

<table>
<thead>
<tr>
<th>Conc. (in ppm)</th>
<th>Injection No.</th>
<th>Peak areas</th>
<th>RSD (Acceptance criteria ≤ 2.0%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1</td>
<td>414905</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>416504</td>
<td>0.838</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>416349</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>520915</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>410940</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>412587</td>
<td></td>
</tr>
</tbody>
</table>

Robustness

Robustness of the method was investigated by varying the instrumental conditions such as flow rate (± 10 %), minor component in mobile phase (± 10 % relative) and column oven temperature (± 5°C). It is concluded that all system suitability parameters are passed as per criteria and the % of RSD of all ingredients were not more than 2.0%. Results were represented in figure-4.11.
Table-4.11: Robustness results.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variation</th>
<th>System suitability</th>
<th>% of RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tailing factor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ornidazole</td>
<td>Ofloxacin</td>
</tr>
<tr>
<td>Standard solution</td>
<td>-</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>+0.1 mL per min</td>
<td>0.9</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>-0.1 mL per min</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Organic solvent ratio</td>
<td>+5%</td>
<td>1.3</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>-5%</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Buffer pH</td>
<td>+0.1 unit</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>-0.1 Unit</td>
<td>1.5</td>
<td>1.6</td>
</tr>
</tbody>
</table>

%RSD: - (%) relative standard deviation.

4.9. SUMMARY AND CONCLUSION

The proposed HPLC method has the advantage of being faster than the two reported HPLC methods used for the simultaneous estimation of Ornidazole, Ofloxacin and Cefixime in their binary mixture. All the proposed procedure was rapid, precise and direct. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The good recoveries obtained in all cases as well as the reliable agreement with the compendial method proved that the proposed methods could be applied and results were observed with satisfactory precision and could be easily used in quality control laboratory for their analysis.
4.10. REFERENCES


193


22. Chalumeau, M.; Tonnelier, S; D’athis, P; Tréluyer, JM; Gendrel, D; Bréart, G; Pons, G; Pediatric Fluoroquinolone Safety Study Investigators, Investigators (June 2003). "Fluoroquinolone Safety in Pediatric Patients: A Prospective, Multicenter, Comparative Cohort Study in France" (PDF). Pediatrics 111 (6 Pt 1): e714. doi:10.1542/peds.111.6.e714. ISSN 0031-4005. PMID 12777590.

c986819 3431736 00Q1 3452229 DEATH DE LEVOFLOXACIN (01/19/00 R.W.
JOHNSON 13 year old male) 3461848 00Q1 SUDDEN DEATH UNEXPLAINED DE
LEVOFLOXACIN (01/19/00 R.W. JOHNSON 13 year old male)

Tablets)" (PDF). USA: FDA.

26. Susan Blank; Julia Schillinger (May 14, 2004). "DOHMH ALERT #8:
Fluoroquinolone-resistant gonorrhea, NYC". USA: New York County Medical

27. Drlica K, Zhao X (1 September 1997). "DNA gyrase, topoisomerase IV, and the

quinolones and novobiocin on calf thymus DNA polymerase alpha primase
complex, topoisomerases I and II, and growth of mammalian lymphoblasts".

norfloxacin and AM-833 in bacteria and mammalian cells". Rev. Infect. Dis 10
(Suppl. 1): S148–S149.

"Effects of ciprofloxacin on eucaryotic pyrimidine nucleotide biosynthesis and
cell growth" (PDF). Antimicrob. Agents Chemother. 31 (5): 774–9. ISSN 0066-
4804.

quinolone antibacterial agents on eucaryotic topoisomerases and related test

toward eukaryotic cells. Identification of topoisomerase II as the primary
13150–3.


48. TsingHua, Determination of ornidazole in plasma by HPLC and study on its pharmacokinetics, Chinese Pharmaceutical Journal 2003