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Research Article

**RP-HPLC METHOD AND ITS VALIDATION FOR ANALYSIS
OF OFLOXACIN AND SATRANIDAZOLE IN BULK AND
PHARMACEUTICAL DOSAGE FORM****Jonnalagadda Sarvani*¹, Dr. Madhusudhan Reddy²**¹Department Of Pharmaceutical Analysis, , Princeton College Of Pharmacy, Pocharam,
Hyderabad, Telangana 500088**Abstract:**

A simple, precise, and accurate Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Ofloxacin and Satranidazole in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using a Phenomenex Luna C18 column (4.6 × 150 mm, 5 µm) with a mobile phase comprising Acetonitrile and Water (55:45 %v/v). The method employed a flow rate of 1.0 mL/min, UV detection at 262 nm, an injection volume of 10 µL, and a run time of 7 minutes. The drugs showed well-resolved, symmetrical peaks with retention times of approximately 2.3 minutes for Ofloxacin and 4.5 minutes for Satranidazole, with no interference from excipients. The method was validated according to ICH Q2(R1) guidelines, demonstrating excellent linearity ($R^2 > 0.999$), accuracy (recovery between 98–102%), precision (RSD < 2%), and specificity. The method showed adequate sensitivity with low limits of detection and quantitation, and robustness was confirmed under slight variations in chromatographic conditions.

Keywords: RP-HPLC, Ofloxacin and Satranidazole, Phenomenex Luna C18, simultaneous estimation, validation.**Corresponding author:****Jonnalagadda Sarvani,**Department of Pharmaceutical Analysis, Princeton college of Pharmacy,
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INTRODUCTION:

Chromatography is a widely used laboratory technique for separating and analyzing components of a mixture based on their differential interactions with a stationary phase and a mobile phase. It is crucial in many fields such as chemistry, biochemistry, pharmaceuticals, and environmental science for purifying, identifying, and quantifying compounds.

The term "chromatography" was coined by the Russian scientist Mikhail Tsvet in 1903, when he developed the technique to separate plant pigments. The process involves a sample being passed through a medium (stationary phase), where the different components of the sample are separated based on their varying affinities for the stationary phase and their solubility in the mobile phase.

Types of Chromatography:

1. **Paper Chromatography:** A simple technique where a liquid sample is separated on a paper strip, typically used for small-scale analysis.
2. **Thin-Layer Chromatography (TLC):** A more advanced technique that uses a thin layer of adsorbent material (like silica gel) on a plate to separate compounds.
3. **Gas Chromatography (GC):** Involves a gaseous mobile phase and is used primarily for separating volatile compounds.
4. **Liquid Chromatography (LC):** Uses a liquid mobile phase to separate components and is often employed in high-performance liquid chromatography (HPLC).
5. **High-Performance Liquid Chromatography (HPLC):** A more sophisticated form of liquid chromatography that offers higher resolution and is commonly used for the analysis of pharmaceuticals, biochemical substances, and environmental samples.
6. **Ion-Exchange Chromatography:** Separates ions and polar molecules based on their charge and affinity for the stationary phase.
7. **Size-Exclusion Chromatography (SEC):** Separates components based on their size, with larger molecules eluting faster than smaller ones.

Principle of Chromatography:

The separation in chromatography occurs due to differences in the distribution of sample components between the stationary phase (often a solid or liquid) and the mobile phase (gas or liquid). The components of the sample travel at different rates, allowing them

to be isolated from each other. The rate at which a compound moves through the system depends on its chemical affinity for the stationary phase, its size, and its solubility in the mobile phase.

Applications of Chromatography:

- Purification of compounds: Isolating pure substances from mixtures.
- Identification and quantification: Analyzing complex mixtures for identification and concentration of components.
- Pharmaceutical analysis: Monitoring drug formulations, detecting impurities, and ensuring quality control.
- Environmental analysis: Detecting pollutants in air, water, and soil.

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. High performance liquid chromatography (HPLC) is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product.[1] The principle is that a solution of the sample is injected into a column of a porous material (stationary phase) and a liquid (mobile phase) is pumped at high pressure through the column. The separation of sample is based on the differences in the rates of migration through the column arising from different partition of the sample between the stationary and mobile phase. Depending upon the partition behaviour of different components, elution at different time takes place. [2] The sample compound with the greater affinity to the stationary layer will travel slower and for a shorter distance in comparison to compounds with less affinity which travel faster and for a longer distance. [3] The High-Performance Liquid Chromatography is more versatile than gas chromatography since (a) it is not limited to volatile and thermally stable samples, and

(b) the choice of mobile and stationary phases is wider. [4] HPLC has numerous advantages like

- Simultaneous Analysis
- High Resolution
- High Sensitivity
- Good repeatability
- Small sample size
- Moderate analysis condition.
- Easy to fractionate the sample and purify. [5]

Classification of HPLC can be done as:

- preparative HPLC and analytical HPLC (based on scale of operation)

- affinity chromatography, adsorption chromatography, size exclusion chromatography, ion exchange chromatography, chiral phase chromatography (based on principle of separation)
- gradient separation and isocratic separation, (based on elution technique)
- normal phase chromatography and reverse phase chromatography (based on modes of operation).[6]

A. Normal phase chromatography:

In normal phase chromatography, mobile phase is non-polar and stationary phase is polar. Hence, the station phase retains the polar analyte. An increase in polarity of solute molecules increases the adsorption capacity leading to an increased elution time. Chemically modified silica (cyanopropyl, aminopropyl and diol) is used as a stationary phase in this chromatography. [7] For example. A typical column has an internal diameter of around 4.6 mm, and a length in the range of 150 to 250 mm. Polar compounds in the mixture that are passed through the column will stick longer to the polar silica than the non-polar compounds. Therefore, the non-polar ones will pass more quickly through the column.[8]

B. RP-HPLC (Reversed phase HPLC):

RP-HPLC has a non-polar stationary phase and polar or moderately polar mobile phase. RP-HPLC is based on the principle of hydrophobic interaction [9]. In a mixture of components those analytes which are relatively less polar will be retained by the non-polar stationary phase longer than those which are relatively more polar. Therefore the most polar component will elute first. [10]

METHOD DEVELOPMENT ON HPLC

A step involved in method development of HPLC is as follows:

1. Understanding the Physicochemical properties of drug molecule.
2. Selection of chromatographic conditions.
3. Developing the approach of analysis.
4. Sample preparations
5. Method optimization
6. Method validation

1. Understanding the Physicochemical properties of drug molecule.

Physicochemical properties of a drug molecule play an important role in method development. For Method development one has to study the physical properties like solubility, polarity, pKa and pH of the drug molecule. Polarity is a physical property of a compound. It helps an analyst, to decide the solvent and composition of the mobile phase. The solubility

of molecules can be explained on the basis of the polarity of molecules. Polar, e.g. water, and nonpolar, e.g. benzene, solvents do not mix. In general, like dissolves like i.e., materials with similar polarity are soluble in each other. The selection of mobile phase or diluents is based on the solubility of analyte. The analyte must be soluble in diluents and must not react with any of its component. pH and pKa plays an important role in HPLC method development. The pH value is defined as the negative of the logarithm to base 10 of the concentration of the hydrogen ion.

$$\text{pH} = -\log_{10}[\text{H}_3\text{O}^+]$$

Selecting a proper pH for ionizable analytes often leads to symmetrical and sharp peaks in HPLC. Sharp, symmetrical peaks are necessary in quantitative analysis in order to achieve low detection limits, low relative standard deviations between injections, and reproducible retention times. [11-12]

1. Selection of chromatographic conditions:

Selection of column:

Selection of the stationary phase/column is the first and the most important step in method development. The development of a rugged and reproducible method is impossible without the availability of a stable, high-performance column. To avoid problems from irreproducible sample retention during method development, it is important that columns be stable and reproducible. A C8 or C18 column made from specially purified, less acidic silica and designed specifically for the separation of basic compounds is generally suitable for all samples and is strongly recommended. [13] Column dimensions, silica substrate properties and bonded stationary phase characteristics are the main ones. The use of silica-based packing is favored in most of the present HPLC columns due to several physical characteristics. [14]

Buffer Selection

Choice of buffer is governed by the pH that is desired. The typical pH range for reversed phase on silica-based packing is pH 2 to 8. It is important that the buffer has a pKa close to the desired pH since buffer controls pH best at their pKa. A rule is to choose a buffer with a pKa value <2 units of the desired mobile phase pH.

General consideration for buffer selection:

1. Phosphate is more soluble in methanol/water than in acetonitrile/water or THF/water.
2. Some salt buffers are hygroscopic and this may lead to changes in the chromatography like increased tailing of basic compounds and possibly selectivity differences.

3. Ammonium salts are generally more soluble in organic/water mobile phases.
4. Trifluoroacetic acid can degrade with time. It is volatile and absorbs at low UV wavelengths.
5. Microbial growth can quickly occur in buffered mobile phases that contain little or no organic modifier at all. The growth accumulates on column inlets and can damage chromatographic performance.
6. At pH greater than 7, phosphate buffer accelerates the dissolution of silica and severely shortens the lifetime of silica-based HPLC columns. If possible, organic buffers should be used at pH greater than 7.
7. Ammonium bicarbonate buffers usually are prone to pH changes and are usually stable for only 24 - 48 hrs. The pH of this mobile phase tends to become more basic due to the release of carbon dioxide.
8. After buffers are prepared, they should be filtered through a 0.2-µm filter.
9. Mobile phases should be degassed. [15]

Buffer Concentration

Generally, a buffer concentration of 10-50 mM is adequate for small molecules. Generally, no more than 50% organic should be used with a buffer. This will depend on the specific buffer as well as its concentration. Phosphoric acid and its sodium or potassium salts are the most common buffer systems for reversed-phase HPLC. Sulfonate buffers can replace phosphonate buffers when analyzing organophosphate compounds. [16]

Isocratic and Gradient Separations:

Isocratic mode of separation includes constant eluent composition; means equilibrium conditions in the column and the actual velocity of compounds moving through the column are constant. The peak capacity is low and the longer the component is retained on the column the wider is the resultant peak. Gradient mode of separation includes significantly increases the separation power of a system mainly due to increase of the apparent efficiency (decrease of the peak width). Peak width varies depending on the rate of the eluent composition variation. In deciding whether a gradient or isocratic would be required an initial gradient run is performed and the ratio between the total gradient time and the difference in the gradient time between the first and last component are calculated. The calculate ratio is <0.25 isocratic is adequate. When the ratio is >0.25 gradient would be adequate [9]

Internal Diameter

The internal diameter (ID) of an HPLC column is an important parameter that influences the detection sensitivity and separation selectivity in gradient elution. It also determines the quantity of analyte that can be loaded into a column. [17]

Particle size

Most traditional HPLC is performed with the stationary phase attached to the outside of small spherical silica particles. These silica particles come in many sizes with 5 µm beads being the most commonly used. The smaller particles usually provide more surface area and better separations but the pressure required for the optimum linear velocity increases by the inverse of the particle diameter squared. Larger particles are used in preparative HPLC where column diameters are in range of 5 cm to >30 cm and for non-HPLC applications such as solid – phase extraction. [18-19]

Pore size

Pore size of column defines an ability of the analyte molecules to penetrate inside the particle and interact with its inner surface [20]

Selection of Mobile Phase:

The mobile phase effects resolution, selectivity and efficiency. Mobile phase composition (or solvent strength) plays an important role in RP-HPLC separation. Acetonitrile (ACN), methanol (MeOH) and tetrahydrofuran (THF) are commonly used solvents in RP-HPLC having low UV cut-off of 190, 205 and 212nm respectively. These solvents are miscible with water. Mixture of acetonitrile and water is the best initial choice for the mobile phase during method development. [21]

Selection of detectors

Detector is a very important part of HPLC. Selection of detector depends on the chemical nature of analyses, potential interference, limit of detection required, availability and/or cost of detector. UVvisible detector is versatile, dual wavelength absorbance detector for HPLC. This detector offers the high sensitivity required for routine UV-based applications to low-level impurity identification and quantitative analysis. Photodiode Array (PDA). Detector offers advanced optical detection for Waters analytical HPLC, preparative HPLC, or LC/MS system solutions. Its integrated software and optics innovations deliver high chromatographic and spectral sensitivity. Refractive index chromatographic and spectral sensitivity, stability and reproducibility, which make this detector the ideal solution for analysis of components with limited or no UV absorption. Multi-wavelength Fluorescence

Detector offers high sensitivity and selectivity fluorescence detection for quantitating low concentrations of target compounds. [22-23]

2. Developing the approach for analysis:

While developing the analytical method on RP-HPLC the first step which is followed is the selections of various chromatographic parameters like selection of mobile phase, selection of column, selection of flow rate of mobile phase, selection of pH of mobile phase. All of these parameters are selected on the basis of trials and followed by considering the system suitability parameters. Typical parameters of system suitability are e.g. retention time should be more than 5 min, the theoretical plates should be more than 2000, the tailing factor should be less than 2, resolution between 2 peaks should be more than 5, % R.S.D. of the area of analyte peaks in standard chromatograms should not be more than 2.0 %.like other. Detection wavelength is usually isobestic point in the case of simultaneous estimation of 2 components. After this the linearity of the drug is studied in order to know the range of concentrations up to which the drug follows the linear pattern. Analysis of the laboratory mixture is also carried out in order to know practicability of developed method for simultaneous estimation. After that analysis of marketed formulation is carried out by diluting the

marketed formulation up to concentration range of linearity. [24-29]

3. Sample preparation:

Sample preparation is an essential part of HPLC analysis, intended to provide a reproducible and homogenous solution that is suitable for injection onto the column. The aim of sample preparation is a sample aliquot that, Is relatively free of interferences, Will not damage the column, and Is compatible with the intended HPLC method that is, the sample solvent will dissolve in the mobile phase without affecting sample retention or resolution. Sample preparation begins at the point of collection, extends to sample injection onto the HPLC column.[30]

4. Method optimization:

Identify the “weaknesses” of the method and optimize the method through experimental design. Understand the method performance with different conditions, different instrument set ups and different samples. [31]

5. Method Validation

Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled. A process of evaluating method performance and demonstrating that it meets a particular .

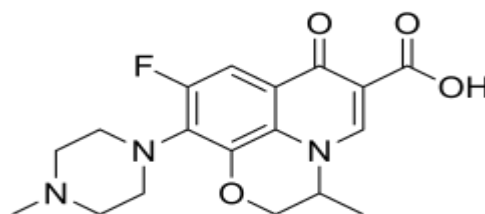
MATERIALS AND METHODS:

OFLOXACIN

Drug: Ofloxacin

Drug Category: Fluoroquinolone Antibiotic

Structure:



Chemical Name / IUPAC Name: (RS)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de][1,4] benzoxazine-6-carboxylic acid

Molecular Formula: C₁₈H₂₀FN₃O₄

Molecular Weight: 361.37 g/mol

Official Pharmacopoeia: USP, BP

PHYSICOCHEMICAL PROPERTIES

Description: Pale yellow to yellow crystalline powder

Solubility: Slightly soluble in water, freely soluble in glacial acetic acid and methanol

Stability: Stable under normal conditions; avoid light and moisture

Storage Conditions: Store at 20–25°C; protect from light

Melting Point: ~270 °C

pKa: ~6.1

Log P: ~ -0.39

PHARMACOKINETIC PROPERTIES

Bioavailability: ~98% orally

Half-life: 4–5 hours

Absorption: Rapid and nearly complete after oral administration

Volume of Distribution: ~1.5–2.7 L/kg

Protein Binding: ~32%

Metabolism: Minimal hepatic metabolism

Excretion: Renal (~75–80% unchanged)

PHARMACODYNAMICS

Mechanism of Action: Inhibits bacterial DNA gyrase and topoisomerase IV → prevents DNA replication and transcription

Therapeutic Indications: UTIs, respiratory tract infections, skin infections, bacterial conjunctivitis

Adverse Reactions: GI upset, CNS effects (dizziness, headache), QT prolongation, tendonitis

Contraindications: Hypersensitivity to fluoroquinolones, children, pregnancy

INTERACTIONS

Drug Interactions: Antacids, iron, and zinc reduce absorption; NSAIDs ↑ seizure risk

Food Interactions: Food may delay absorption slightly but does not affect extent

DRUG FORMULATION

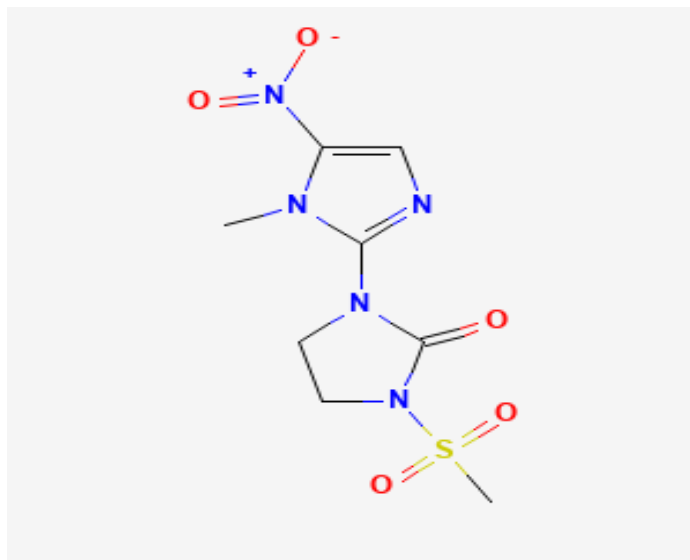
S. No	Drug Name	Label Claim	Brand Name	Company
1	Ofloxacin	200 mg Tablet	Zanocin	Ranbaxy

SATRANIDAZOLE

Drug: Satranidazole

Drug Category: Nitroimidazole derivative, Antiprotozoal, Antibacterial

Structure:



Chemical Name / IUPAC Name: 1-[1-(ethanesulfonyl)-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-yl]-1H-imidazole

Molecular Formula: C₈H₁₀N₄O₄S

Molecular Weight: 258.25 g/mol

Official Pharmacopoeia: Indian Pharmacopoeia (IP)

PHYSICOCHEMICAL PROPERTIES

Description: Yellow crystalline powder

Solubility: Slightly soluble in water; soluble in DMSO, ethanol

Stability: Stable in dry form; sensitive to light and moisture

Storage Conditions: Store at 15–30°C in a dry, light-resistant container

Melting Point: ~160–165 °C

pKa: ~2.6

Log P: ~0.5

PHARMACOKINETIC PROPERTIES

Bioavailability: ~100% (oral)

Half-life: 4–6 hours

Absorption: Rapid after oral administration

Volume of Distribution: Not well defined

Protein Binding: Moderate (~30–40%)

Metabolism: Hepatic (partially via reduction of nitro group)

Excretion: Renal and fecal (as metabolites and unchanged drug)

PHARMACODYNAMICS

Mechanism of Action: Disrupts DNA helical structure in anaerobic organisms → inhibits nucleic acid synthesis

Therapeutic Indications: Amoebiasis, giardiasis, trichomoniasis, bacterial vaginosis

Adverse Reactions: Metallic taste, nausea, dizziness, headache

Contraindications: Hypersensitivity, neurological disorders, alcohol consumption during therapy

INTERACTIONS

Drug Interactions: Disulfiram-like reaction with alcohol; ↑ warfarin effect

Food Interactions: No major effect

DRUG FORMULATION

S. No	Drug Name	Label Claim	Brand Name	Company
1	Satranidazole	300 mg Tablet	Zintec	FDC Ltd.

RESULTS AND DISCUSSION:

Trails

Trail 1:

Mobile phase : ACN: Water (80:20%v/v)

Column : Symmetry C18 (4.6 ×150mm, 5µm particle size) Make; waters

Flow rate : 0.8ml/min

Wavelength : 245 nm

Column temp : 40°C

Injection Volume : 10 µl

Run time : 4 minutes

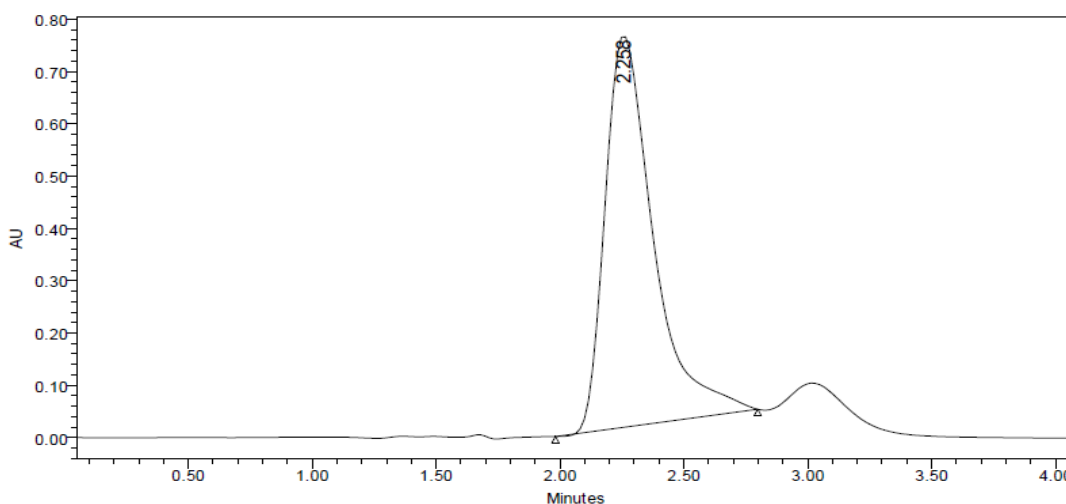


Figure7.1: Chromatogram for Trail 1

Table7.1: Peak results for Trail 1

S.No	Peak Name	R _t	Area	Height	USP Resolution	USP Tailing	USP Plate count
1	Ofloxacin	2.258	1256365	753652		0.86	785

Observation: In a separation of Ofloxacin and Satranidazole peak was obtained only for one compound because there may be less solubility. So, we go for further trails.

Trail 2:

Mobile phase : Water: Methanol (40:60v/v)
 Column : Zodiac C18 (4.6×250mm) 5μ
 Flow rate : 0.8 ml/min
 Wavelength : 245 nm
 Column temp : 40°C
 Injection Volume : 8 μl
 Run time : 2 minutes

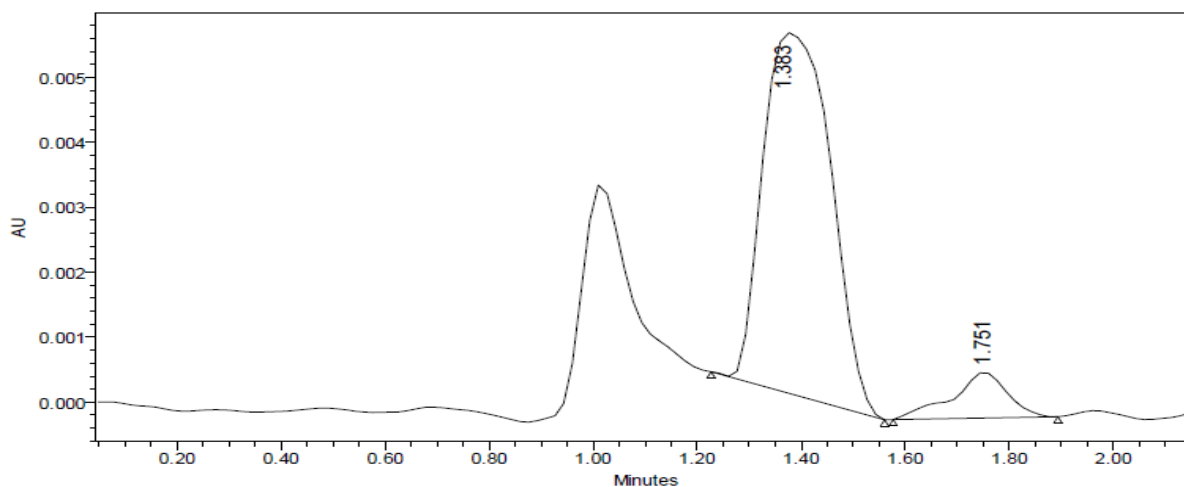


Figure7.2: Chromatogram for trail 2

Table7.2: Peak results for trail 2

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ofloxacin	1.383	5632548	5689		1.23	658
2	Satranidazole	1.751	6958	878	1.9	0.83	895

Observation: The separations of two compounds were obtained but improper separation is obtained and resolution is not good. So, we go for further trails.

Trail 3:

Mobile phase : Methanol: TEA Buffer pH 3.2 (40:60% v/v)
 Column : Zodiac C18 (4.6×250mm 5μm)
 Flow rate : 1.0 ml/min
 Wavelength : 245 nm
 Column temp : 40°C
 Injection Volume : 10 μl
 Run time : 10 minutes

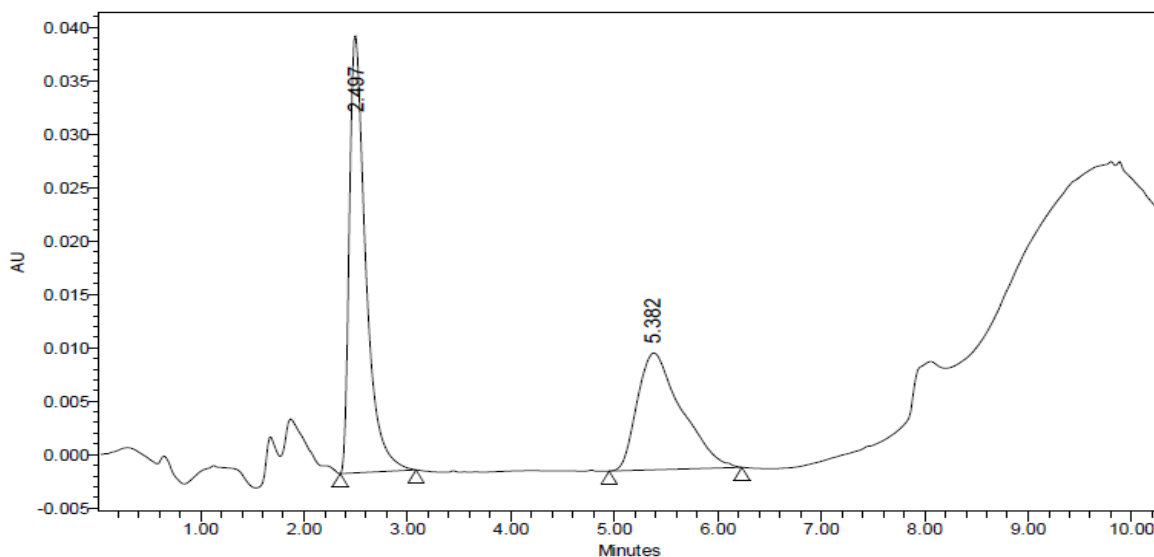


Figure7.3: - Chromatogram for trail 3

Table7.3: - peak results for trail 3

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ofloxacin	2.497	456582	40865		2.5	1365
2	Satranidazole	5.382	33254	10784	1.39	3.5	698

Observation: The separations of two compounds were obtained but base line noise was very high, peak symmetry was not available. We go for further trail as the void peaks are obtained.

Trail 4:

Mobile phase : Acetonitrile: Phosphate Buffer (pH-3.6) (30:70 v/v)
 Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
 Flow rate : 1 ml/min
 Wavelength : 245 nm
 Column temp : 40°C
 Injection Volume : 10 µl
 Run time : 7 minutes

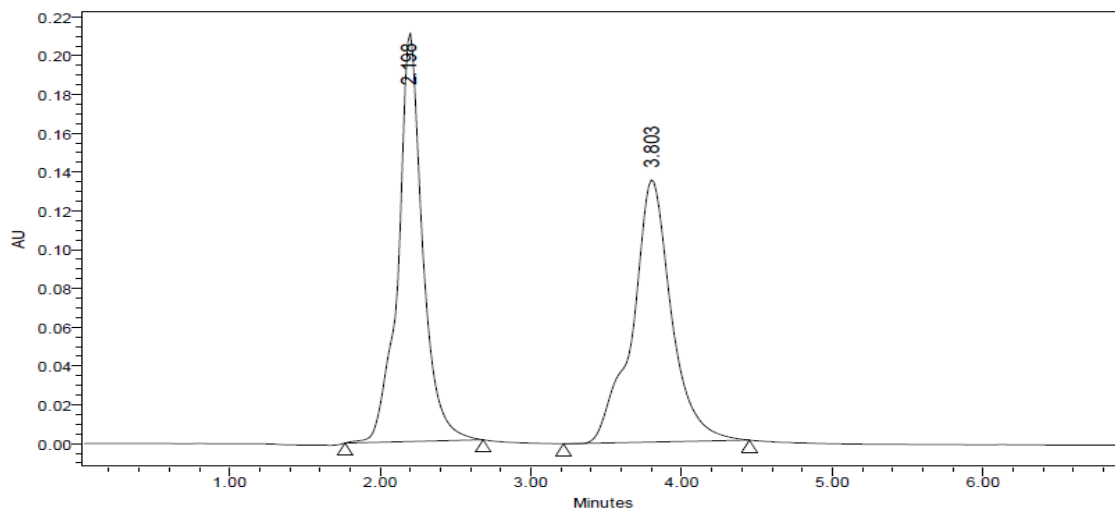


Figure7.4: - Chromatogram for trail 4

Table7.4: - Peak results for trail 4

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ofloxacin	2.198	2458478	212541		1.0	1564
2	Satranidazole	3.803	2465814	136534	3.02	1.0	1452

Observation: After repeating the same conditions got proper peaks, base line is good and we can go for repeatability. Therefore separations of two peaks, base line, peak symmetry, resolution are proper.

Trail 5 (Optimized Chromatogram) (Standard)

Column : Phenomenex Luna C18 (4.6 x 150mm, 5 μ m)
 Mobile phase : Acetonitrile and water (55:45 %v/v)
 Flow rate : 1ml/min
 Wavelength : 262 nm
 Injection volume : 10 μ l
 Run time : 7 min

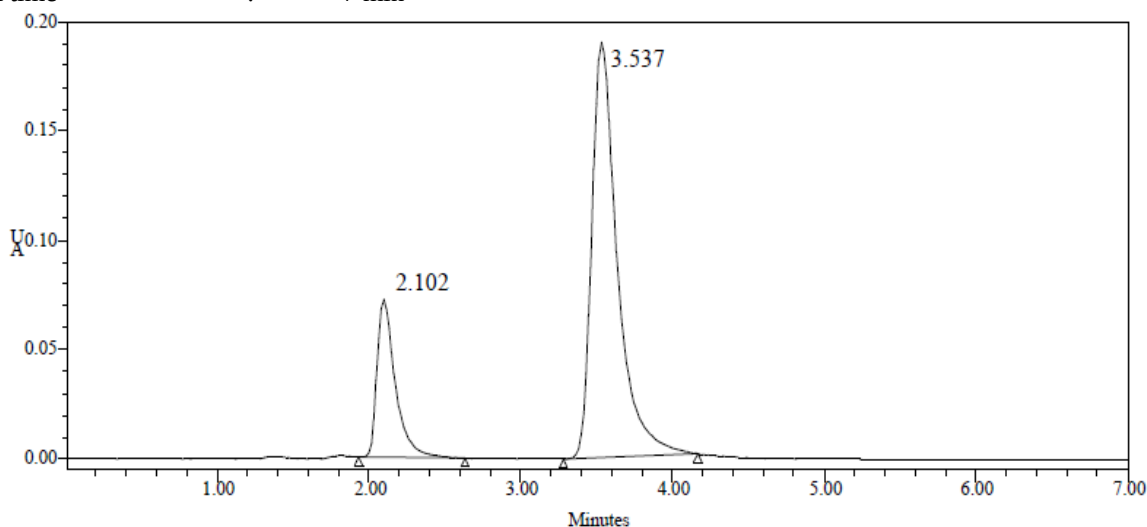


Fig7.5:- Optimized Chromatogram

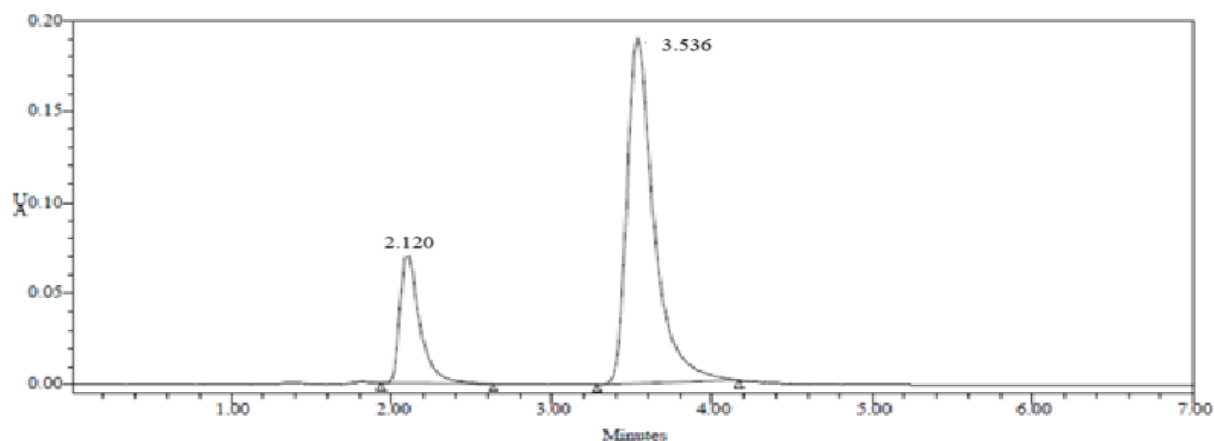
Table7.5: - Peak results for Optimized Chromatogram

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ofloxacin	2.102	765789	69584		0.97	5587.0
2	Satranidazole	3.537	2532158	190049	2.97	1.26	5398.0

Observation: From the above chromatogram it was observed that the Ofloxacin and Satranidazole peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

Column : Phenomenex Luna C18 (4.6 x 150mm, 5 μ m)
 Mobile phase : Acetonitrile and water (45:55 %v/v)
 Flow rate : 1ml/min
 Wavelength : 250 nm
 Injection volume : 10 μ l
 Run time : 7 min

**Figure7.6:- Optimized Chromatogram (Sample)****Table7.6: Optimized Chromatogram (Sample)**

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ofloxacin	2.120	775684	13124		0.99	6365.0
2	Satranidazole	3.536	2658478	937405	5.06	1.23	7458.0

Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.

- It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION**Blank:**

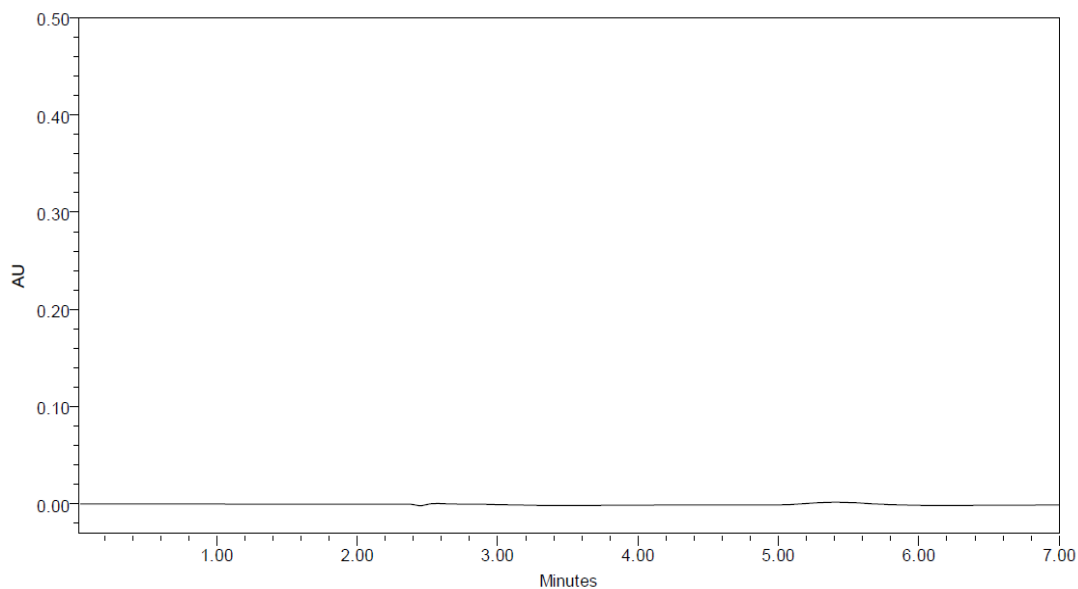


Fig7.7: Chromatogram showing blank (mobile phase preparation)

System suitability:

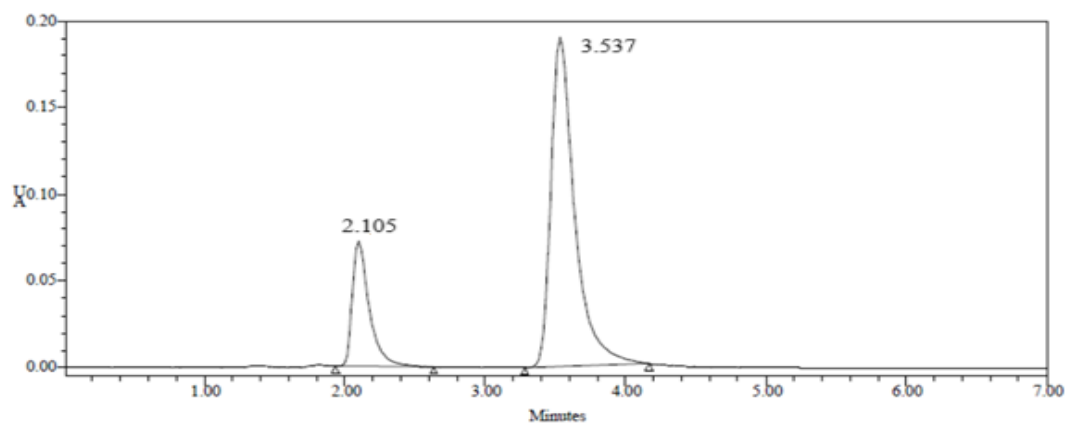


Fig7.8: Chromatogram showing injection -1

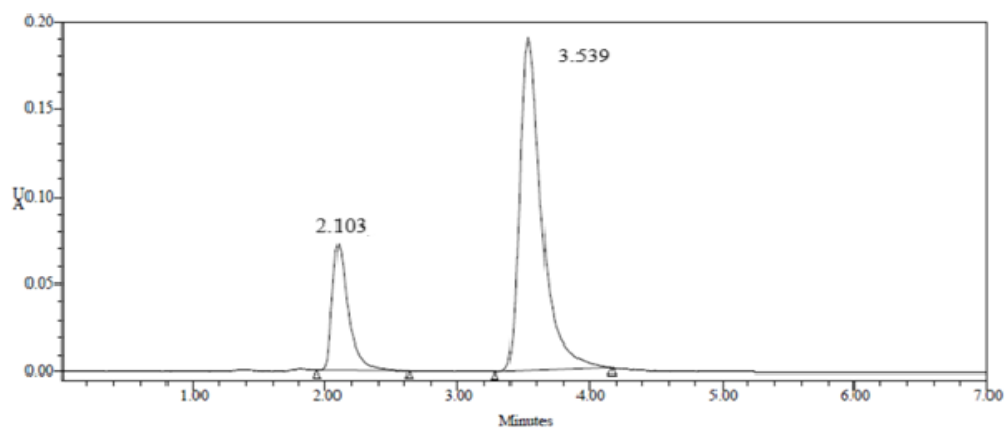


Fig7.9: Chromatogram showing injection -2

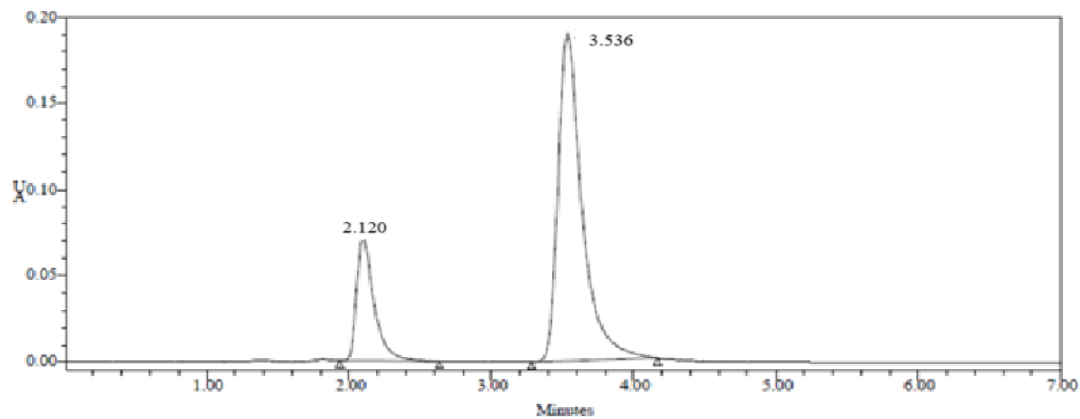
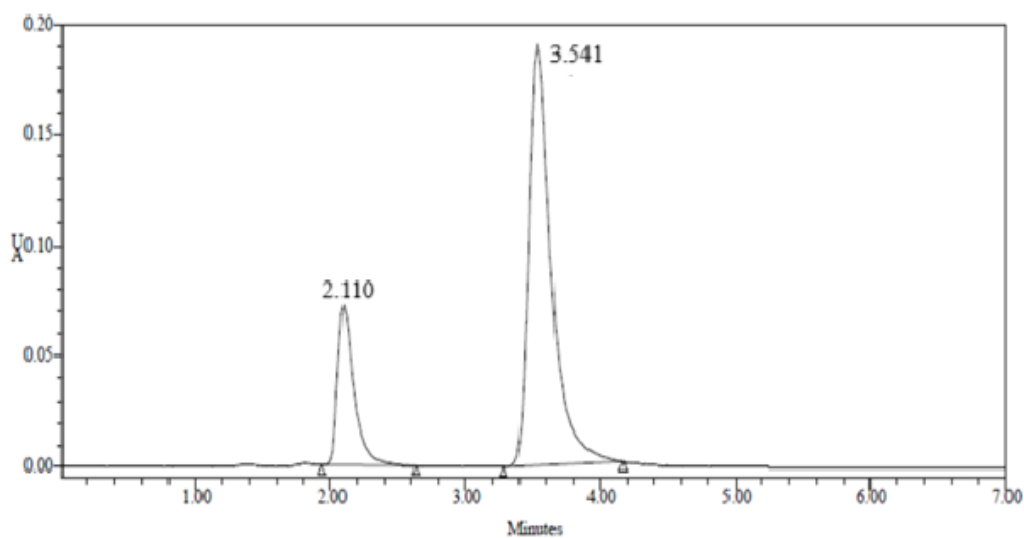
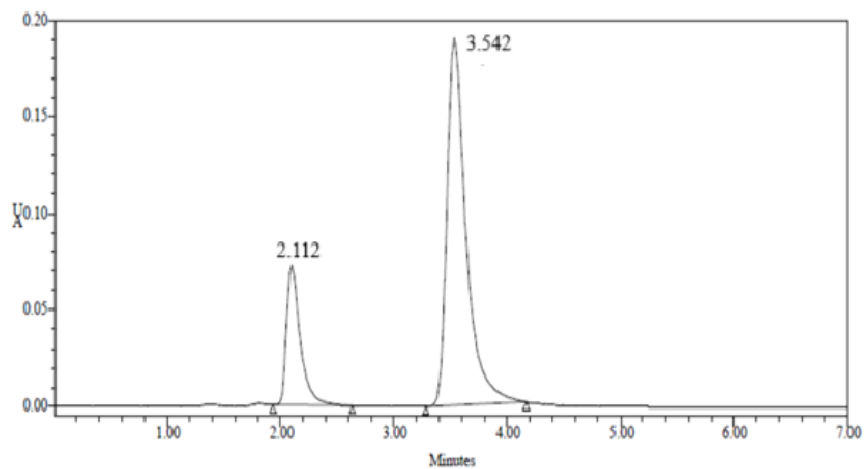
**Fig7.10: Chromatogram showing injection -3****Fig7.11: Chromatogram showing injection -4****Fig7.12: Chromatogram showing injection -5**

Table7.7: Results of system suitability for Ofloxacin

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ofloxacin	2.105	765843	69587	5589	1.9
2	Ofloxacin	2.103	766594	69854	5576	1.6
3	Ofloxacin	2.120	765487	70211	5658	1.6
4	Ofloxacin	2.110	765928	69213	5642	1.7
5	Ofloxacin	2.112	765426	69558	5685	1.6
Mean			765855.6			
Std. Dev			466.6522			
% RSD			0.060932			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table7.8: Results of system suitability for Satranidazole

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Satranidazole	3.537	2534658	190058	5365	1.2	2.07
2	Satranidazole	3.539	2536854	190052	5348	1.4	2.05
3	Satranidazole	3.536	2535879	190078	5389	1.5	2.0
4	Satranidazole	3.541	2533564	190035	5347	1.6	2.01
5	Satranidazole	3.542	2534214	190085	5364	1.6	2.01
Mean			2535034				
Std. Dev			1183.309				
% RSD			0.046678				

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of

components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitated Ofloxacin and Satranidazole in drug product.

Assay (Standard):

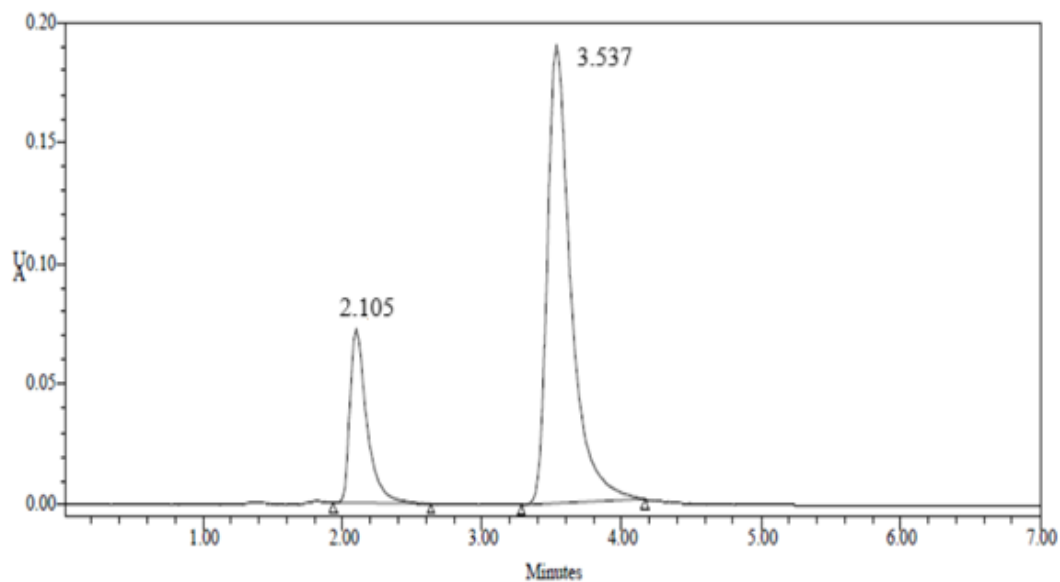


Fig-7.13: Chromatogram showing assay of standard injection -1

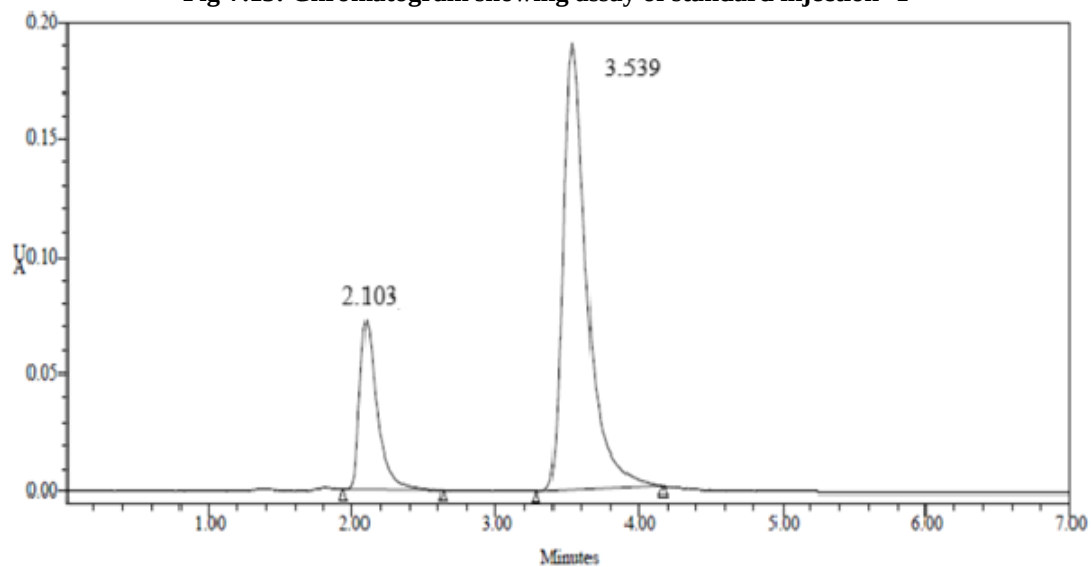


Fig-7.14: Chromatogram showing assay of standard injection -2

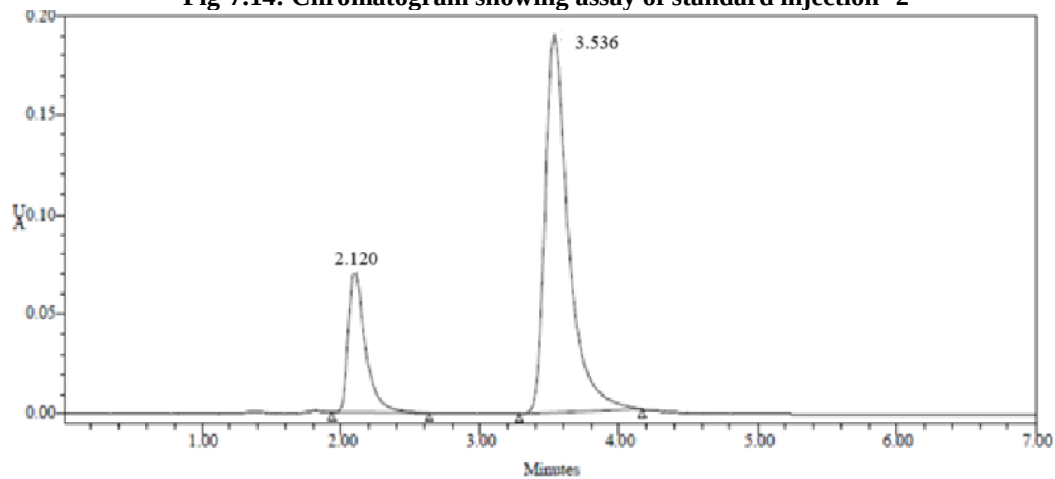
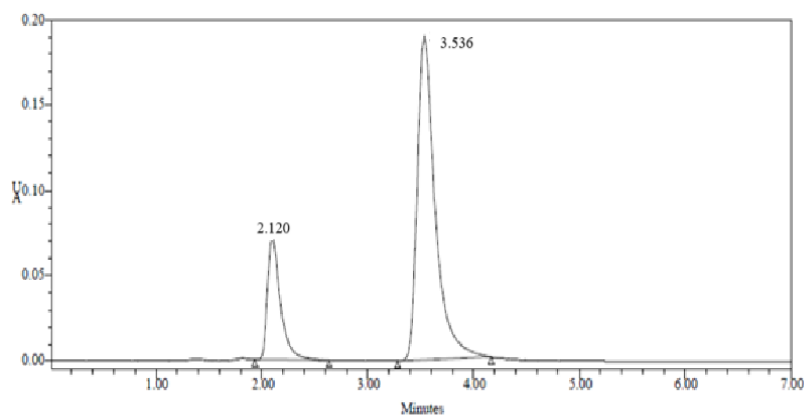
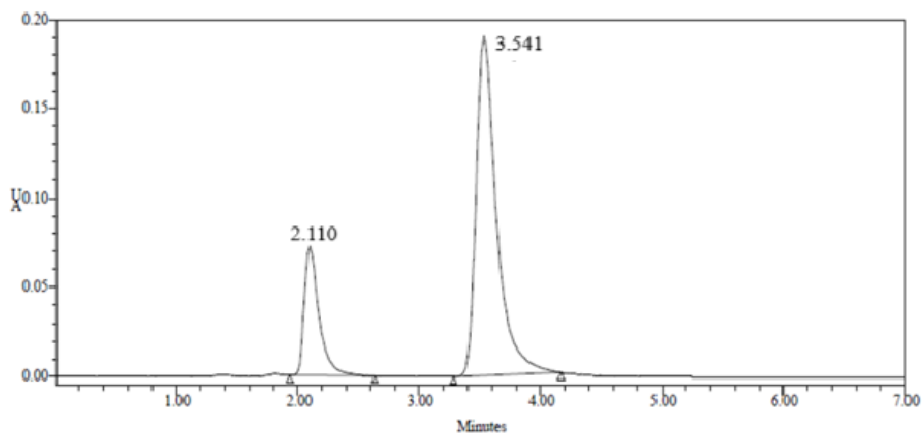


Fig-7.15: Chromatogram showing assay of standard injection -3

Table7.9:- Peak results for assay standard

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Ofloxacin	2.105	759868	71255		1.7	5689	1
2	Satranidazole	3.537	2458754	215654	2.04	1.6	5362	1
3	Ofloxacin	2.103	759458	72541		1.7	5748	2
4	Satranidazole	3.539	2465885	226565	2.00	1.6	5452	2
5	Ofloxacin	2.120	759245	72584		1.7	5584	3
6	Satranidazole	3.536	2489578	221542	2.04	1.6	5456	3

Assay (Sample):**Fig7.16: Chromatogram showing assay of sample injection-1****Fig7.17: Chromatogram showing assay of sample injection-3**

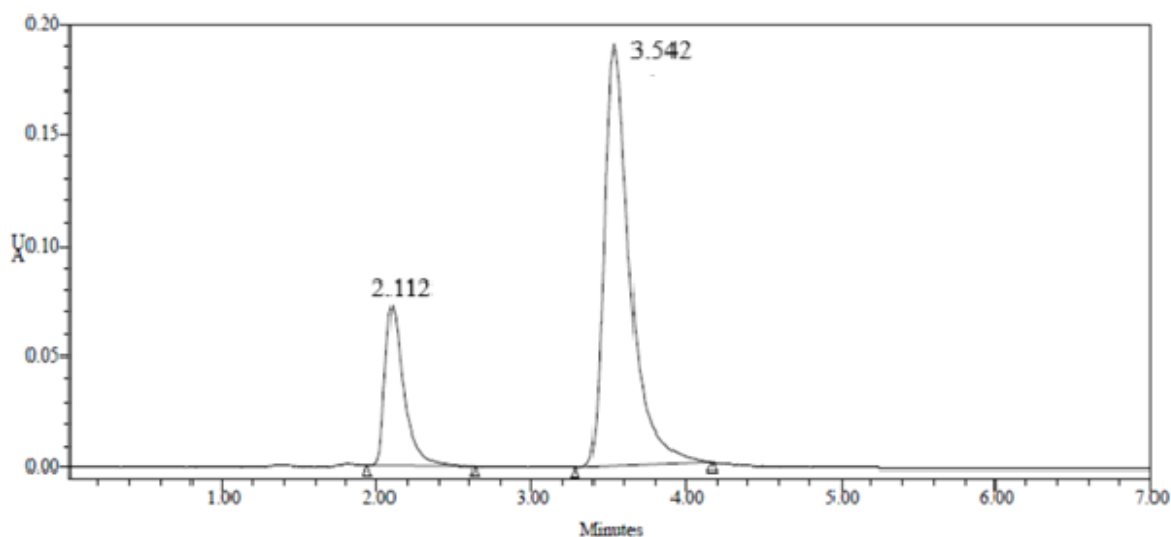


Fig7.18: Chromatogram showing assay of sample injection-3

Table7.10: Peak results for Assay sample

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Ofloxacin	2.120	756985	68958		0.98	7253	1
2	Satranidazole	3.536	2569856	198564	2.06	1.23	8836	1
3	Ofloxacin	2.110	758745	69857		1.05	6530	2
4	Satranidazole	3.541	2598654	195682	2.04	0.99	7270	2
5	Ofloxacin	2.112	756848	69588		1.7	7586	3
6	Satranidazole	3.542	2587454	192541	2.04	1.6	8371	3

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Ofloxacin and Satranidazole in pharmaceutical dosage form was found to be 99.8%.

LINEARITY

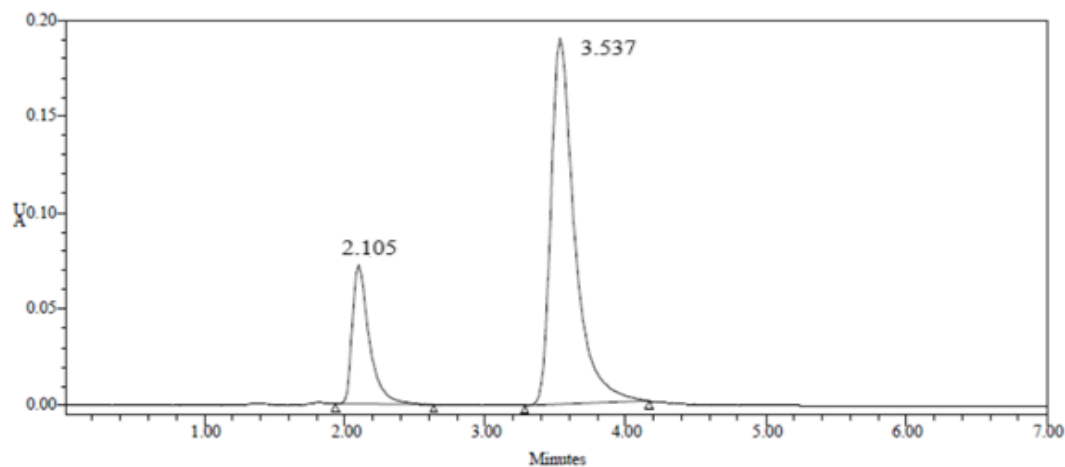


Figure7.19:- Chromatogram for linearity concentration-15µg/ml of Ofloxacin & 10 µg/ml of Satranidazole

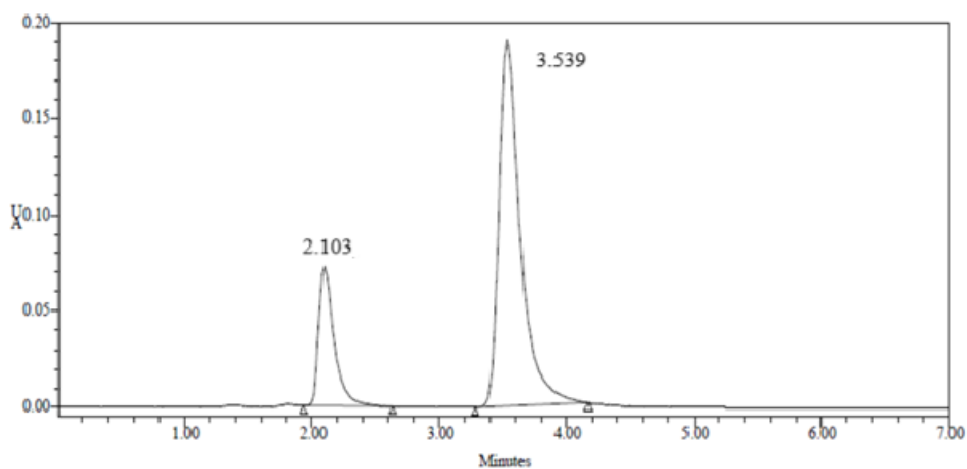


Figure7.20:- Chromatogram for linearity concentration-30 µg/ml of Ofloxacin & 20 µg/ml of Satranidazole

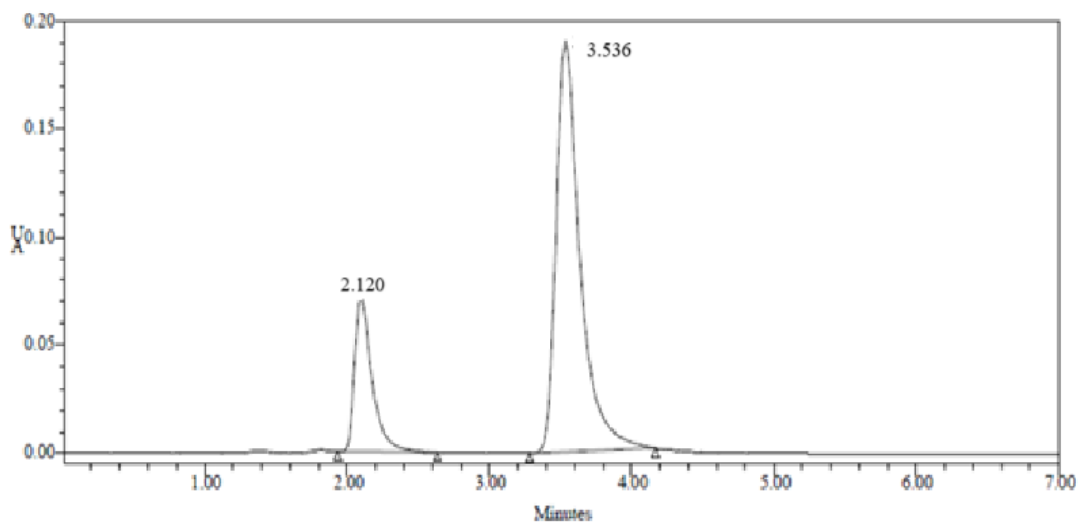


Figure-7.21: chromatogram for linearity concentration-45 µg/ml of Ofloxacin & 30 µg/ml of Satranidazole

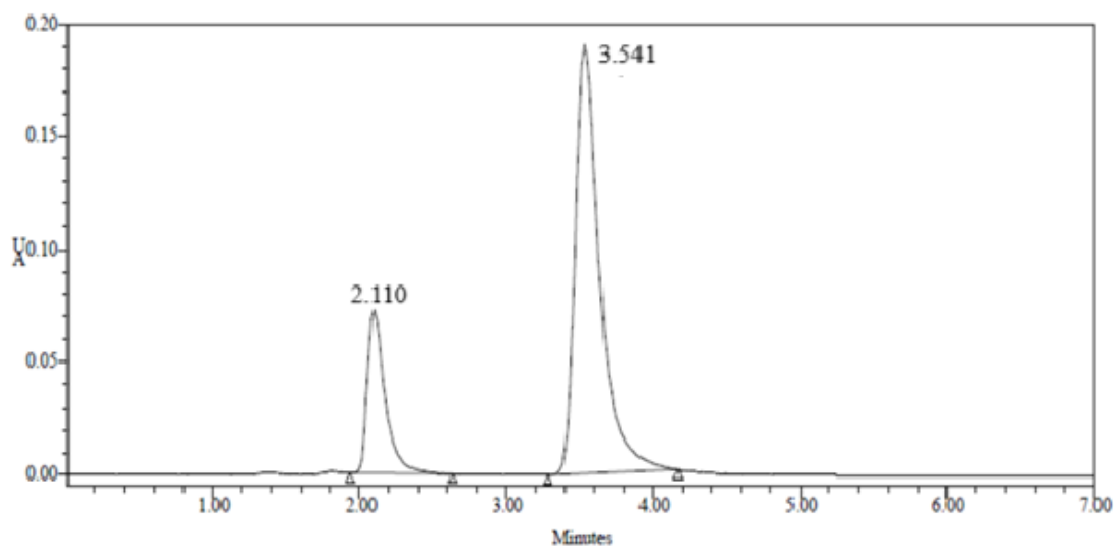


Figure-7.22: chromatogram for linearity concentration-60 µg/ml of Ofloxacin & 40 µg/ml of Satranidazole

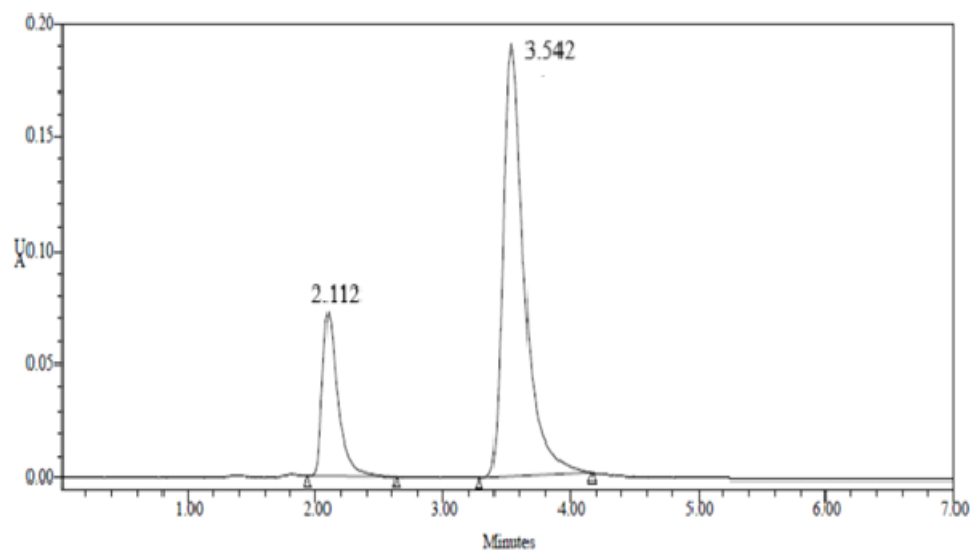


Figure-7.23: Chromatogram for linearity concentration-75 µg/ml of Ofloxacin & 50 µg/ml of Satranidazole

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Ofloxacin:

Concentration µg/ml	Average Peak Area
0	0
15	205035
30	381239
45	561128
60	740162
75	909922

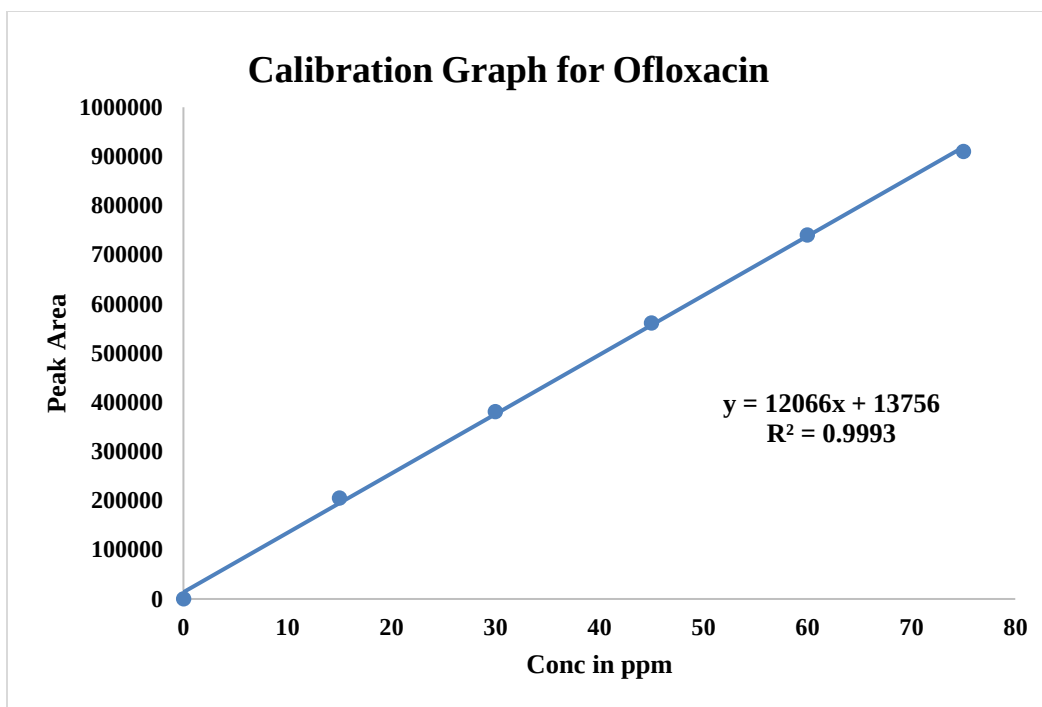


Figure-7.24: Calibration Graph for Ofloxacin

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Ofloxacin is a straight line.

$$Y = mx + c$$

$$\text{Slope (m)} = 12066$$

$$\text{Intercept (c)} = 13756$$

$$\text{Correlation Coefficient (r)} = 0.999$$

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 13756. These values meet the validation criteria.

Satranidazole

Concentration µg/ml	Average Peak Area
0	0
10	757881
20	1457881
30	2132457
40	2901811
50	3501811

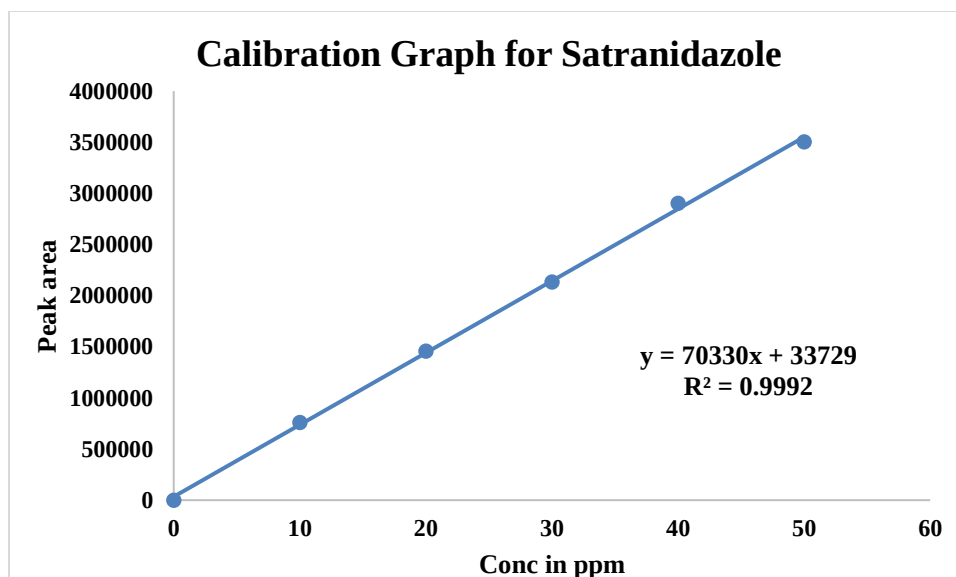


Figure-7.25: Calibration Graph for Satranidazole

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Satranidazole is a straight line.

$$Y = mx + c$$

$$\text{Slope (m)} = 70330$$

$$\text{Intercept (c)} = 33729$$

$$\text{Correlation Coefficient (r)} = 0.999$$

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 33729. These values meet the validation criteria.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

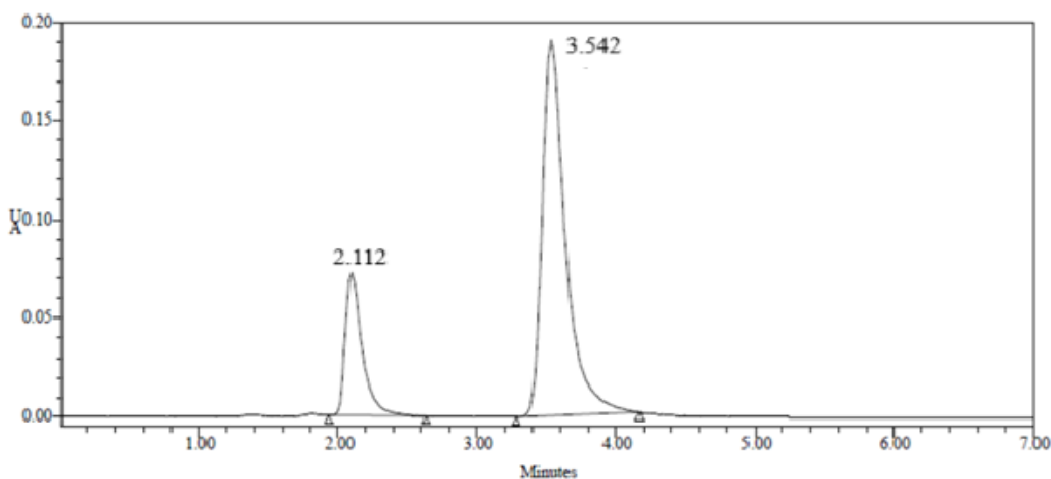


Fig-7.26: Chromatogram showing precision injection -1

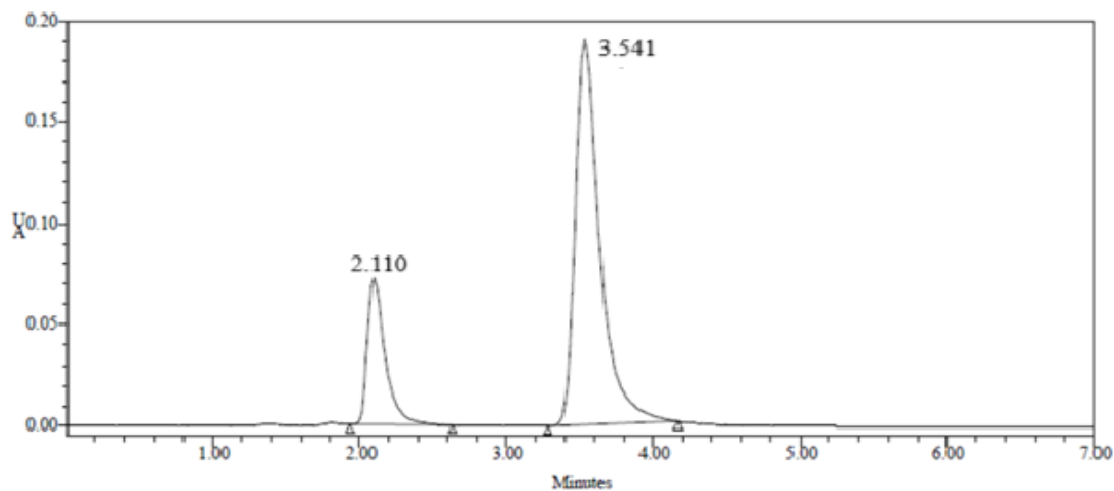


Fig-7.27: Chromatogram showing precision injection -2

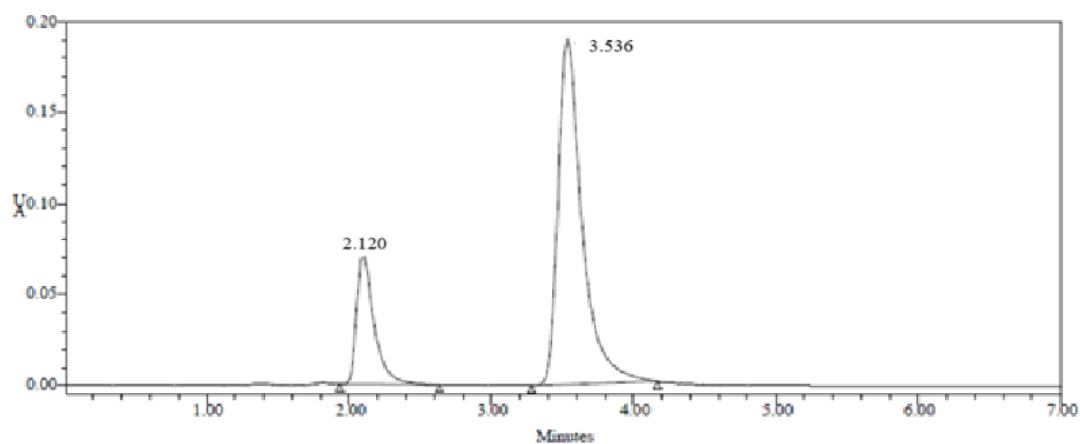


Fig-7.28: Chromatogram showing precision injection -3

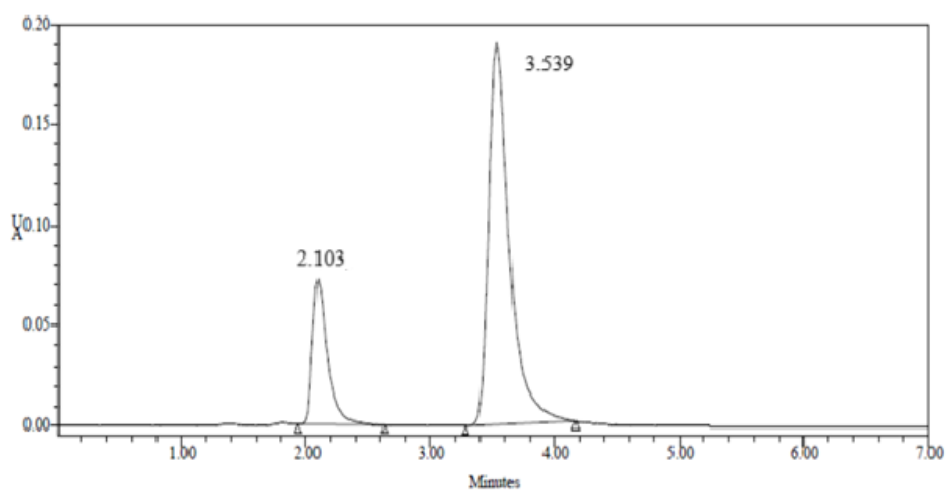


Fig-7.29: Chromatogram showing precision injection -4

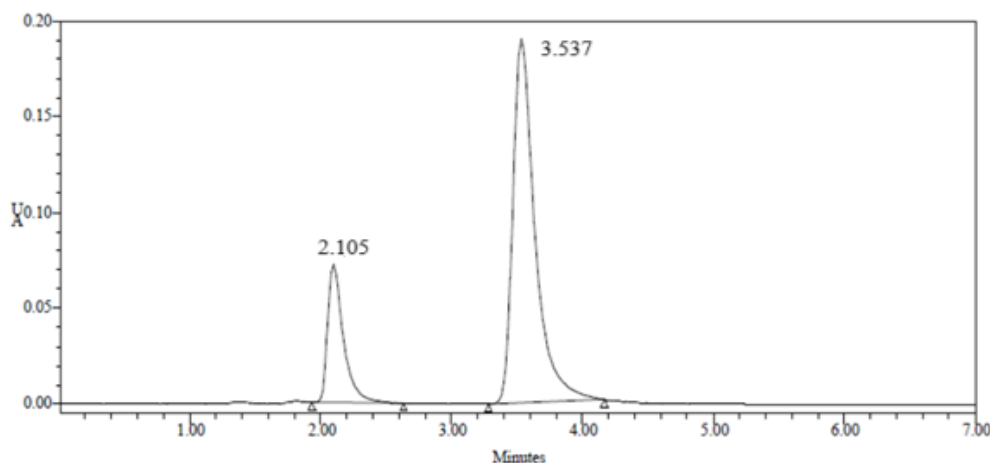


Fig-7.30: Chromatogram showing precision injection -5

Table 7.11: Results of Repeatability for Ofloxacin:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ofloxacin	2.112	766854	702564	5685	1.6

CONCLUSION:

A simple, precise, and accurate Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Ofloxacin and Satranidazole in bulk and pharmaceutical dosage forms. The chromatographic separation was carried out using a Phenomenex Luna C18 column (4.6 × 150 mm, 5 µm) with a mobile phase consisting of Acetonitrile and Water in the ratio of 55:45 %v/v. The flow rate was maintained at 1.0 mL/min with detection at 262 nm using a UV detector. The injection volume was 10 µL, and the total run time was 7 minutes. Under the optimized conditions, both drugs were well separated with sharp, symmetrical peaks and satisfactory retention times—approximately 2.3 minutes for Ofloxacin and 4.5 minutes for Satranidazole—ensuring no interference from excipients or impurities.

The method was validated according to ICH Q2(R1) guidelines, demonstrating excellent linearity ($R^2 > 0.999$) over the tested concentration range. Accuracy was confirmed through recovery studies, which showed values within 98–102%, while precision studies revealed relative standard deviations (RSD) of less than 2%, indicating good repeatability and reproducibility. The method was also found to be specific, as no interference from formulation

components was observed, and sensitive, with appropriately low LOD and LOQ values. Furthermore, robustness testing confirmed that minor changes in chromatographic conditions did not significantly affect the results.

In conclusion, the proposed RP-HPLC method is validated, reliable, and suitable for routine quality control analysis of Ofloxacin and Satranidazole in both bulk drugs and combined dosage forms. The short analysis time, high accuracy, and clear resolution make it highly effective for simultaneous estimation in pharmaceutical settings.

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