



CODEN [USA]: IAJ PBB

ISSN : 2349-7750

**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES**

SJIF Impact Factor: 7.187

<https://doi.org/10.5281/zenodo.23279363>Available online at: <http://www.iajps.com>

Research Article

**VALIDATED RP-HIGH-PERFORMANCE LIQUID
CHROMATOGRAPHY METHOD FOR ESTIMATION OF
RANITIDINE AND METOCLOPRAMIDE IN BULK AND
TABLET DOSAGE FORM****Dharavath Ahalya*, Dr.K. Hariprasad**Department Of Pharmaceutical Analysis, Princeton College Of Pharmacy, Pocharam,
Hyderabad, Telangana 500088.**Abstract:**

A validated, precise, and accurate High-Performance Liquid Chromatography (HPLC) method was developed for the simultaneous estimation of Ranitidine and Metoclopramide in bulk and tablet dosage forms. The chromatographic analysis was performed using an X-Bridge C18 column (4.6 × 150 mm, 5 µm) at ambient temperature, with a mobile phase consisting of Methanol and Water (65:35 v/v). The flow rate was maintained at 1 mL/min, and detection was carried out at a wavelength of 260 nm. The injection volume was 10 µL, and the total run time was 14 minutes. The method provided effective resolution of both analytes with sharp and distinct peaks, indicating successful separation. Validation of the method was conducted as per ICH guidelines, demonstrating excellent linearity, accuracy, precision, specificity, and robustness. The proposed method is suitable for routine quality control and simultaneous quantification of Ranitidine and Metoclopramide in pharmaceutical preparations.

Keywords: RP-HPLC, Ranitidine and Metoclopramide, X-Bridge C18 column, simultaneous estimation, validation.

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Please cite this article in press **Dharavath Ahalya et al Validated Rp-High Performance Liquid Chromatography Method For Estimation Of Ranitidine And Metoclopramide In Bulk And Tablet Dosage For.,. Indo Am. J. P. Sci, 2026; 13(10).**

INTRODUCTION:

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the *mobile phase*, which carries it through a structure holding another material called the *stationary phase*. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.^[1]

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.^[2]

Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights. Because of these differences, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into mobile phase, and leave the system faster [3].

Based on this approach three components form the basis of the chromatography technique.

- Stationary phase: This phase is always composed of a “solid” phase or “a layer of a liquid adsorbed on the surface a solid support”.
- Mobile phase: This phase is always composed of “liquid” or a “gaseous component.”
- Separated molecules

The type of interaction between stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other. Chromatography methods based on partition are very effective on separation, and identification of small molecules as amino acids, carbohydrates, and fatty acids. However, affinity chromatographies (ie. ion-exchange chromatography) are more effective in the separation of macromolecules as nucleic acids, and proteins. Paper chromatography is used in the separation of proteins, and in studies related to protein synthesis; gas-liquid chromatography is utilized in the separation of alcohol, ester, lipid,

and amino groups, and observation of enzymatic interactions, while molecular-sieve chromatography is employed especially for the determination of molecular weights of proteins. Agarose-gel chromatography is used for the purification of RNA, DNA particles, and viruses ^[4].

Stationary phase in chromatography, is a solid phase or a liquid phase coated on the surface of a solid phase. Mobile phase flowing over the stationary phase is a gaseous or liquid phase. If mobile phase is liquid, it is termed as liquid chromatography (LC), and if it is gas then it is called gas chromatography (GC). Gas chromatography is applied for gases, and mixtures of volatile liquids, and solid material. Liquid chromatography is used especially for thermal unstable, and non-volatile samples ^[5].

The purpose of applying chromatography which is used as a method of quantitative analysis apart from its separation, is to achieve a satisfactory separation within a suitable time interval. Various chromatography methods have been developed to that end. Some of them include column chromatography, thin-layer chromatography (TLC), paper chromatography, gas chromatography, ion exchange chromatography, gel permeation chromatography, high-pressure liquid chromatography, and affinity chromatography ^[6].

- ✚ Column chromatography
- ✚ Ion-exchange chromatography
- ✚ Gel-permeation (molecular sieve) chromatography
- ✚ Affinity chromatography
- ✚ Paper chromatography
- ✚ Thin-layer chromatography
- ✚ Gas chromatography
- ✚ Dye-ligand chromatography
- ✚ Hydrophobic interaction chromatography
- ✚ Pseudoaffinity chromatography
- ✚ High-pressure liquid chromatography (HPLC)

High-pressure liquid chromatography (HPLC)

Using this chromatography technique it is possible to perform structural, and functional analysis, and purification of many molecules within a short time. This technique yields perfect results in the separation, and identification of amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules. In HPLC, mobile phase passes through columns under 10–400 atmospheric pressure, and with a high (0.1–5 cm/sec) flow rate. In this technique, use of small particles, and application of high pressure on the rate of solvent flow increases

separation power, of HPLC and the analysis is completed within a short time.

Essential components of a HPLC device are solvent depot, high- pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled

Essential components of a HPLC device are solvent depot, high- pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled with the aid of a computerized system, and material is accrued.

Types of HPLC

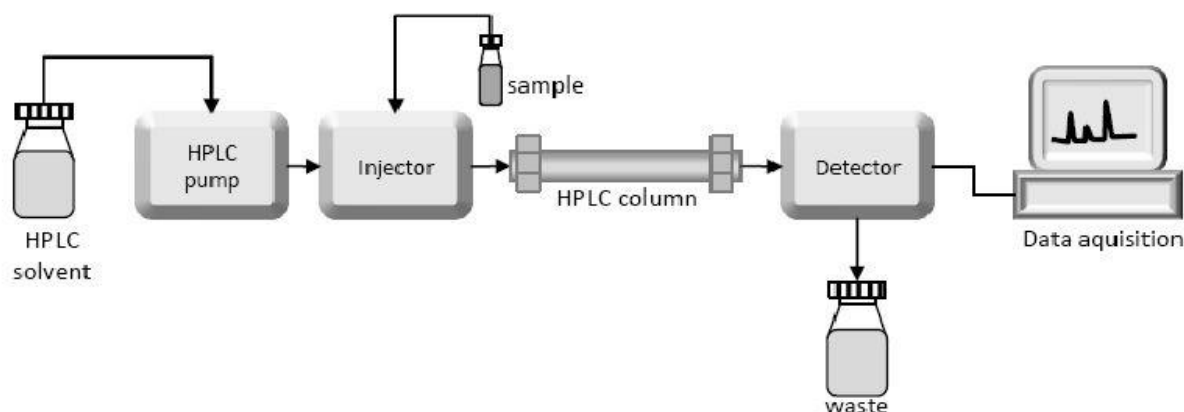
There are following variants of HPLC, depending upon the phase system (stationary) in the process:

1. Normal Phase HPLC

This method separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these.

Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

Instrumentation of HPLC



As shown in the schematic diagram in Figure above, HPLC instrumentation includes a pump, injector, column, detector and integrator or acquisition and display system. The heart of the system is the column where separation occurs.

1. Solvent Reservoir

Mobile phase contents are contained in a glass reservoir. The mobile phase, or solvent, in HPLC is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample.

2. Pump

A pump aspirates the mobile phase from the solvent reservoir and forces it through the system's column and detector. Depending on a number of factors including column dimensions, particle size

2. Reverse Phase HPLC

The stationary phase is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more nonpolar the material is, the longer it will be retained.

3. Size-exclusion HPLC

The column is filled with material having precisely controlled pore sizes, and the particles are separated according to their molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later.

4. Ion-Exchange HPLC

The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples.

The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.

of the stationary phase, the flow rate and composition of the mobile phase, operating pressures of up to 42000 kPa (about 6000 psi) can be generated.

3. Sample Injector

The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).

4. Columns

Columns are usually made of polished stainless steel, are between 50 and 300 mm long and have an internal diameter of between 2 and 5 mm. They are

commonly filled with a stationary phase with a particle size of 3–10 μm .

Columns with internal diameters of less than 2 mm are often referred to as microbore columns. Ideally the temperature of the mobile phase and the column should be kept constant during an analysis.

5. Detector

The HPLC detector, located at the end of the column detects the analytes as they elute from the chromatographic column. Commonly used

detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical detectors.

6. Data Collection Devices

Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store and reprocess chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.

Applications of HPLC

The information that can be obtained by HPLC includes resolution, identification and quantification of a compound. It also aids in chemical separation and purification. The other applications of HPLC include:

- **Pharmaceutical Applications**
 1. To control drug stability.
 2. Tablet dissolution study of pharmaceutical dosages form.
 3. Pharmaceutical quality control.
- **Environmental Applications**
 1. Detection of phenolic compounds in drinking water.
 2. Bio-monitoring of pollutants.
- **Applications in Forensics**
 1. Quantification of drugs in biological samples.
 2. Identification of steroids in blood, urine etc.
 3. Forensic analysis of textile dyes.
 4. Determination of cocaine and other drugs of abuse in blood, urine etc.
- **Food and Flavour**
 1. Measurement of Quality of soft drinks and water.
 2. Sugar analysis in fruit juices.
 3. Analysis of polycyclic compounds in vegetables.
 4. Preservative analysis.
- **Applications in Clinical Tests**
 1. Urine analysis, antibiotics analysis in blood.
 2. Analysis of bilirubin, biliverdin in hepatic disorders.
 3. Detection of endogenous Neuropeptides in extracellular fluid of brain etc.

METHOD DEVELOPMENT

Analytical method development is considered as a critical process in pharmaceuticals. Availability of the different types of columns, operating parameters, mobile phase composition, diluent and pH values make it critical to develop an analytical method. A good analytical method should be simple, used column, mobile phase and buffer should be common. It can be done easily step by step. Following are the common HPLC method development steps.

1. Selection of HPLC Analytical Method
2. Selection of Chromatographic Conditions

3. Parameter Optimization

1. Selection of HPLC Analytical Method: First of all consult the literature that is available on the product. It will help you to understand the nature of the product that will help to select the different parameters.

A. Sample Preparation: Select method to prepare the sample according to its solubility, filtration requirements, extraction requirements or other special requirements to make a clear solution of HPLC analysis.

B. Chromatography: Reverse phase chromatography is used for most of the samples but when acidic or basic molecules are present in the sample then reverse phase ion suppression (for weak acid or base) or reverse phase ion pairing (for strong acid or base) should be used. The stationary phase should be C18 bonded. Normal phase is used for low or medium polarity analyte especially when it is required to separate the product isomers. Choose cyano bonded phase for normal phase separations. Ion exchange chromatography is best to use for inorganic anion or cation analysis. If analyte has higher molecular weight than size exclusion chromatography is the best to use.

C. Gradient/Isotonic HPLC: Gradient HPLC is helpful in the analysis of complex samples having a number of components. It will help to get higher resolution than isotonic HPLC having constant peak width while in isotonic HPLC peak width increases with the retention time. Gradient HPLC has great sensitivity, especially for the products having longer retention time.

D. Column Size: 100-150 mm columns are used for most of the samples. It reduces the method development and analysis time for the sample. Bigger columns are used for complex samples those take more time in separation. Initially, a flow rate should be kept between 1 and 1.5 ml/min and column particle size should be between 3 and 5 μm .

E. HPLC Detectors: If the analyte has chromophores that enable the compound to be detected by UV than it is better to use UV detector. It is always better to use a UV detector than others. Fluorescence and electrochemical detectors should be used for trace analysis. Samples having high

concentration should be analyzed using refractive index detectors.

F. Wavelength: λ_{max} of the sample has the greatest sensitivity to the UV light. It detects the sample components that have chromophores. A wavelength above 200 nm gives greater sensitivity than the lower wavelengths. Wavelengths lower than 200 nm gives more noise, therefore, it should be avoided. Related: Analytical Method Development Protocol.

2. Selection of Chromatographic Conditions: After selection of analytical method, different chromatographic conditions are selected. The flow of the analytes through the column depends upon the concentration of the solvent in the mobile phase. The concentration of solvent is generally used to control the retention time. Mobile phase pH and ion pairing reagents also affect the retention time of the sample. Samples having a large number of components are analyzed using the gradient to avoid the large retention time while the samples containing one or two components are analyzed on an isotonic system.

3. Parameter Optimization: After taking the same sample runs some parameters including column dimensions, particle size, run time and flow rate are optimized. It is done to get the best resolution and minimum run time. After proper optimization of the analysis method, it is validated to ensure the consistency of the analytical method. Analytical method validation is now done mandatory by all regulatory authorities.

Performance calculations

Computing the accompanying qualities (which VAL be incorporated in a custom report) used to get to general framework execution.

1. Relative retention
2. Theoretical plates
3. Capacity factor

4. Resolution

5. Peak asymmetry

6. Plates per meter

Relative retention (Selectivity):

$$= (t_2 - t_a) / (t_1 - t_a)$$

Theoretical plates:

$$n = 16 (t / W)^2$$

Capacity factor:

$$K' = (t_2 / t_a) - 1$$

Resolution:

$$R = 2 (t_2 - t_1) / (W_2 + W_1)$$

Peak asymmetry:

$$T = W_{0.05} / 2f$$

Plates per meter:

$$N = n / L$$

$$\text{HETP: } L/n$$

Where,

t_2 = Retention time of the second peak measured from point of injection.

t_1 = Retention time of the first peak measured from point of injection.

t_a = Retention time of an inert peak not retained by the column, measured from point of injection.

n = Theoretical plates.

t = Retention time of the component.

W = Width of the base of the component peak using tangent method.

K' = Capacity factor.

R = Resolution between a peak of interest (peak 2) and the peak preceding it

W_2 = Width of the base of component peak 2.

W_1 = Width of the base of component peak 1.

T = Peak asymmetry, or tailing factor.

$W_{0.05}$ = Distance from the leading edge to the tailing edge of the peak, measured at a point 5 % of the peak height from the baseline.

f = Distance from the peak maximum to the leading edge of the peak.

MATERIALS AND METHODS:

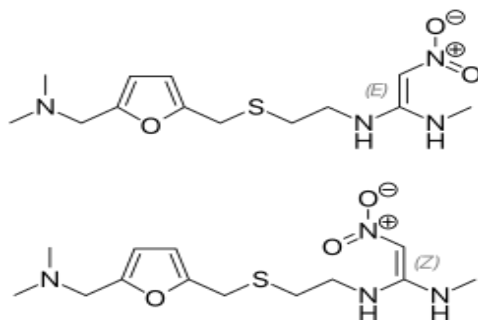
RANITIDINE

Drug: Ranitidine

Synonym: Zantac

Drug category: H2 Receptor Antagonist

Structure:



Chemical name/ Nomenclature / IUPAC Name:

N-[2-[[[5-[(dimethylamino) methyl] furan-2-yl] methyl] thio] methyl] thiazol-4-yl] methyl] acetamide

Molecular Formula: $C_{13}H_{22}N_4O_3S$

Molecular Weight: 314.41 gm/mole

Official Pharmacopoeia: USP

PHYSICOCHEMICAL PROPERTIES:

Description (Physical State): Off-white to pale yellow crystalline powder

Solubility: Soluble in water and methanol; slightly soluble in ethanol

Stability: Stable under normal conditions

Storage Conditions: Store at room temperature (15-30°C) in a tightly closed container

Dosage: Typically 150 mg to 300 mg daily, depending on the condition

Melting point: 138-140 °C

pKa (strongest acidic): 7.0

Log P: 0.6

PHARMACOKINETIC PROPERTIES:

Bioavailability: Approximately 50%

Half-life: 2-3 hours

Absorption: Rapidly absorbed from the gastrointestinal tract

Volume of Distribution: 1-2 L/kg

Protein binding: 15%

Metabolism: Minimal hepatic metabolism

Metabolites: Inactive metabolites

Time of peak action: 1-3 hours

Excretion: Approximately 30% via urine (as unchanged drug and metabolites)

Adverse effects/Side effects: Gastrointestinal disturbances, headache, dizziness

PHARMACODYNAMICS:

Mechanism of action: Inhibits gastric acid secretion by blocking H₂ receptors on parietal cells

Therapeutic efficacy/ Indications: Treats peptic ulcers, gastroesophageal reflux disease (GERD), and Zollinger-Ellison syndrome

Adverse reactions: Rare, but may include allergic reactions

Contraindications: Hypersensitivity to ranitidine

METOCLOPRAMIDE

Drug: Metoclopramide

Synonym: Reglan

Drug category: Antiemetic and Prokinetic Agent

Structure:

Chemical name/ Nomenclature / IUPAC Name:

4-amino-5-chloro-2-methoxy-N-[2-(1-pyrrolidinyl)ethyl]benzamide

DRUG FORMULATION:

Molecular Formula: C₁₄H₂₂ClN₃O₂

Molecular Weight: 270.80 gm/mole

Official Pharmacopoeia: USP

PHYSICOCHEMICAL PROPERTIES:

Description (Physical State): White to off-white crystalline powder

Solubility: Soluble in water; slightly soluble in ethanol

Stability: Stable under normal conditions

Storage Conditions: Store at room temperature (15-30°C) in a tightly closed container

Dosage: Typically 10 mg to 15 mg taken 30 minutes before meals and at bedtime

Melting point: 146-148 °C

pKa (strongest acidic): 8.0

Log P: 1.2

PHARMACOKINETIC PROPERTIES:

Bioavailability: Approximately 80% (due to first-pass metabolism)

Half-life: 5-6 hours

Absorption: Rapidly absorbed from the gastrointestinal tract

Volume of Distribution: 3-4 L/kg

Protein binding: 30%

Metabolism: Primarily hepatic (via CYP2D6)

Metabolites: Inactive metabolites

Time of peak action: 1-2 hours

Excretion: Approximately 90% via urine (as metabolites)

Adverse effects/Side effects: Drowsiness, fatigue, restlessness, and extrapyramidal symptoms

PHARMACODYNAMICS:

Mechanism of action: Increases gastrointestinal motility and accelerates gastric emptying; also acts as an antiemetic

Therapeutic efficacy/ Indications: Used to treat nausea, vomiting, and gastroparesis

Adverse reactions: Extrapyramidal symptoms, tardive dyskinesia with long-term use

Contraindications: Gastrointestinal obstruction, perforation, or hemorrhage; hypersensitivity to metoclopramide

S. No	Drug Name	Label Claim	Brand Name	Company
1	Ranitidine and Metoclopramide	150 mg / 10 mg	Various	Various Manufacturers

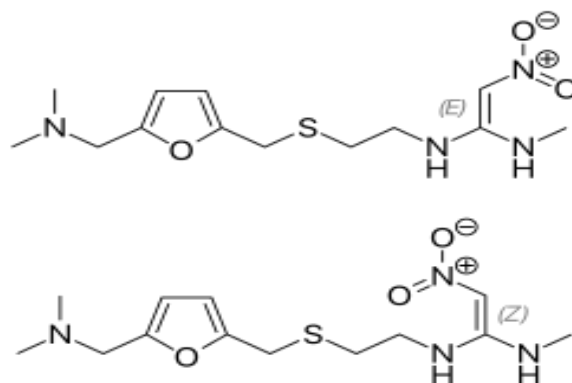
RESULTS AND DISCUSSION:**RANITIDINE**

Drug: Ranitidine

Synonym: Zantac

Drug category: H₂ Receptor Antagonist

Structure:

**Chemical name/ Nomenclature / IUPAC Name:**

N-[2-[[[5-[(dimethylamino) methyl] furan-2-yl] methyl] thio] methyl] thiazol-4-yl] methyl] acetamide

Molecular Formula: C₁₃H₂₂N₄O₃S

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Protein binding: 15%

Metabolism: Minimal hepatic metabolism

Metabolites: Inactive metabolites

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Excretion: Approximately 30% via urine (as unchanged drug and metabolites)

Adverse effects/Side effects: Gastrointestinal disturbances, headache, dizziness

PHARMACODYNAMICS:

Mechanism of action: Inhibits gastric acid secretion by blocking H₂ receptors on parietal cells

Therapeutic efficacy/ Indications: Treats peptic ulcers, gastroesophageal reflux disease (GERD), and Zollinger-Ellison syndrome

Adverse reactions: Rare, but may include allergic reactions

Contraindications: Hypersensitivity to ranitidine

METOCLOPRAMIDE

Drug: Metoclopramide

Synonym: Reglan

Drug category: Antiemetic and Prokinetic Agent

Structure:

Chemical name/ Nomenclature / IUPAC Name:

4-amino-5-chloro-2-methoxy-N-[2-(1-pyrrolidinyl)ethyl]benzamide

Molecular Formula: C₁₄H₂₂ClN₃O₂

Molecular Weight: 270.80 gm/mole

Official Pharmacopoeia: USP

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Description (Physical State): White to off-white crystalline powder

Solubility: Soluble in water; slightly soluble in ethanol

Stability: Stable under normal conditions

Storage Conditions: Store at room temperature (15-30°C) in a tightly closed container

Dosage: Typically 10 mg to 15 mg taken 30 minutes before meals and at bedtime

Melting point: 146-148 °C

pKa (strongest acidic): 8.0

Log P: 1.2

PHARMACOKINETIC PROPERTIES:

Bioavailability: Approximately 80% (due to first-pass metabolism)

Half-life: 5-6 hours

Absorption: Rapidly absorbed from the gastrointestinal tract

Volume of Distribution: 3-4 L/kg

Protein binding: 30%

Metabolism: Primarily hepatic (via CYP2D6)

Metabolites: Inactive metabolites

Time of peak action: 1-2 hours

Excretion: Approximately 90% via urine (as metabolites)

Adverse effects/Side effects: Drowsiness, fatigue, restlessness, and extrapyramidal symptoms

PHARMACODYNAMICS:

Mechanism of action: Increases gastrointestinal motility and accelerates gastric emptying; also acts as an antiemetic

Therapeutic efficacy/ Indications: Used to treat nausea, vomiting, and gastroparesis

Adverse reactions: Extrapyramidal symptoms, tardive dyskinesia with long-term use

Contraindications: Gastrointestinal obstruction, perforation, or hemorrhage; hypersensitivity to metoclopramide

DRUG FORMULATION:

S. No	Drug Name	Label Claim	Brand Name	Company
1	Ranitidine and Metoclopramide	150 mg / 10 mg	Various	Various Manufacturers

CONCLUSION:

Trial 1:

Mobile phase : Methanol + Acetonitrile (60:40%v/v)
 Column : Symmetry ODS C18 (4.6 ×150mm, 5µm particle size)
 Flow rate : 0.8ml/min
 Wavelength : 260 nm
 Column temp : Ambient
 Sample Temp : Ambient
 Injection Volume : 10 µl
 Run time : 10 minutes

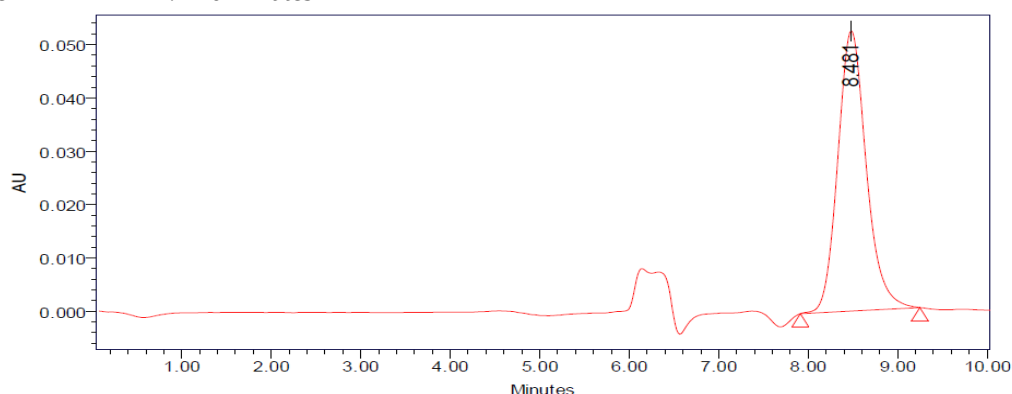


Figure-7.1: Chromatogram for trail 1

Table-7.1: peak results for trail 1

S.No	Peak Name	R _t	Area	Height	USP Resolution	USP Tailing	USP Plate count
1	Metoclopramide	8.481	1153246	535242		1.03	2452

Observation:

This trial shows only one sample peak in the chromatogram and does not shows resolution. so more trials were required for obtaining good peaks.

Trial 2:

Mobile phase : Water: Methanol (55:45 v/v)
 Column : Zodiac C18 (4.6×250mm) 5µ Particle size
 Flow rate : 1.0 ml/min
 Wavelength : 260 nm
 Column temp : Ambient
 Sample Temp : Ambient
 Injection Volume : 10 µl
 Run time : 8 minutes

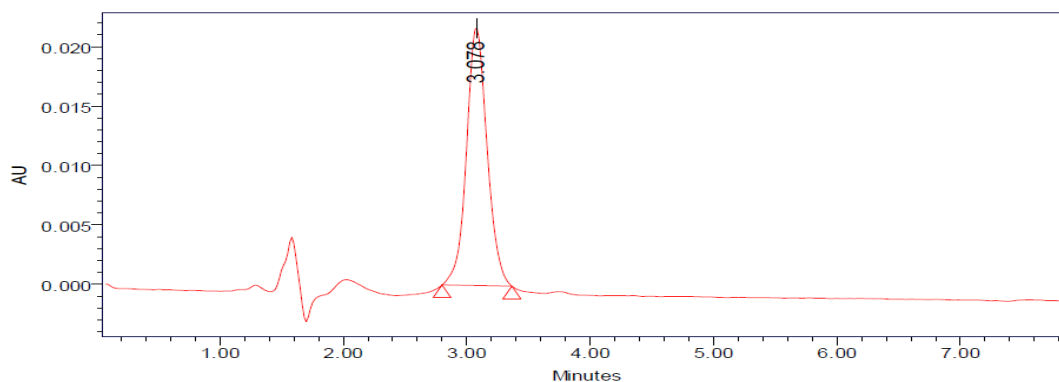


Figure-7.2: Chromatogram for trail 2
Table-7.2: peak results for trail 2

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ranitidine	3.078	3653215	23653		1.03	1897

Observation:

This trial show improper separation of sample peaks, very less plate count, resolution and show improper baseline in the chromatogram, so more trials were required for obtaining good peaks.

Trail 3:

Mobile phase : Acetonitrile: Water (60:40% v/v)
 Column : Zodiac C18 (4.6×250mm 5μm)
 Flow rate : 1.0 ml/min
 Wavelength : 260 nm
 Column temp : Ambient
 Sample Temp : Ambient
 Injection Volume : 10μl
 Run time : 9 minutes

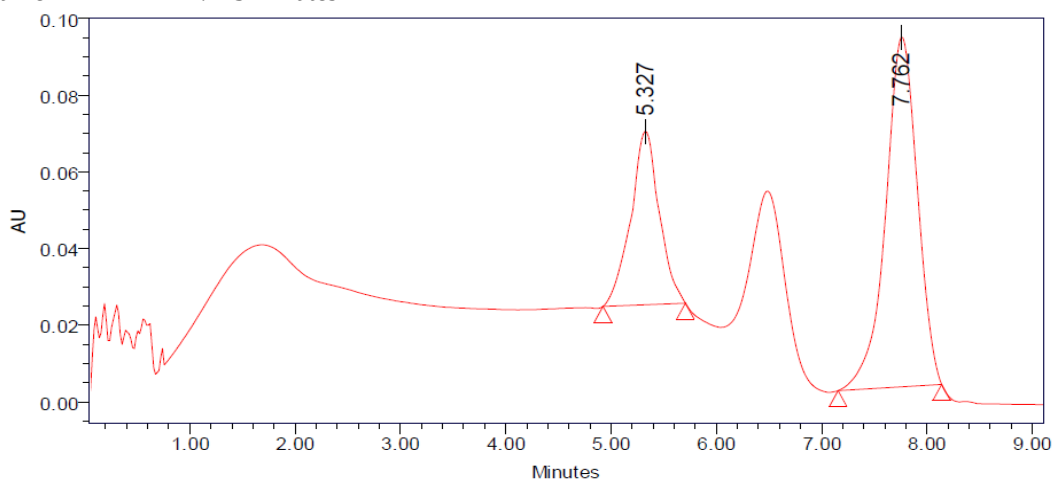


Figure7.3: Chromatogram for trail 3

Table7.3: peak results for trail 3

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ranitidine	5.327	875846	44752		0.98	1786
2	Metoclopramide	7.762	189547	86973	4.22	0.83	2655

Observation:

This trial shows improper baseline and show less plate count in the chromatogram, so more trials were required for obtaining peaks.

Trial 4:

Mobile phase : ACN: Methanol (70:30% v/v)
 Column : Xterra C18 5 μ m (4.6 $\times$ 250mm)
 Flow rate : 1.0 ml/min
 Wavelength : 260 nm
 Column temp : Ambient
 Sample Temp : Ambient
 Injection Volume : 1.0 μ l/min
 Run time : 10 minutes

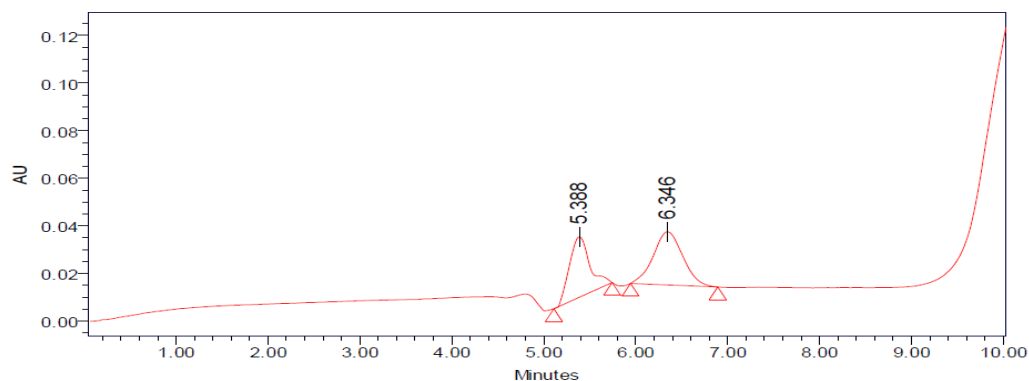


Figure7.4: Chromatogram for trail 4

Table7.4: peak results for trail 4

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ranitidine	5.388	385476	26585		0.98	2155
2	Metoclopramide	6.346	512455	29242	1.15	1.02	1588

Observation:

This trial shows improper separation of sample peaks and baseline, show very less plate count in the chromatogram, so more trials were required for obtaining peaks.

Trial 5:

Mobile phase : ACN: Phosphate Buffer pH-3.2 (60:40v/v)
 Column : Phenomenex Luna C18 (4.6 $\times$ 250mm) 5 μ m
 Flow rate : 1 ml/min
 Wavelength : 260 nm
 Column temp : Ambient
 Sample Temp : Ambient
 Injection Volume : 10 μ l
 Run time : 10 minutes

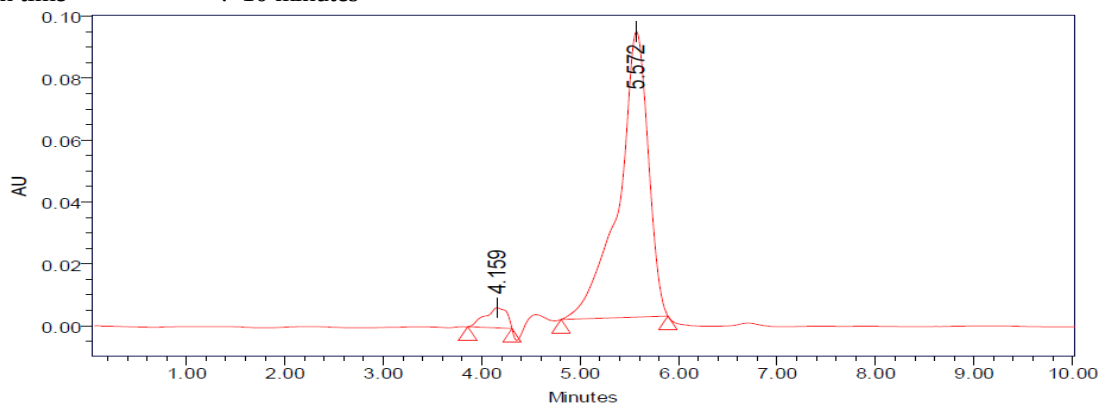


Figure7.5: Chromatogram for trail 5

Table7.5: peak results for trail 5

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ranitidine	4.159	126586	6699		0.87	975
2	Metoclopramide	5.572	2154148	98966	1.66	0.93	2696

Observation:

This trial shows improper separation sample peaks, baseline and show very less plate count in the chromatogram. So it's required more trials to obtain good peaks.

Trial 6 (Optimized Chromatogram) (Standard)

Mobile phase : Methanol and water (65:35 v/v)
 Column : X- bridge column C18 (4.6 x 150mm, 5µm)
 Flow rate : 1 ml/min
 Wavelength : 260 nm
 Column temp : Ambient
 Injection Volume : 10 µl
 Run time : 14 minutes

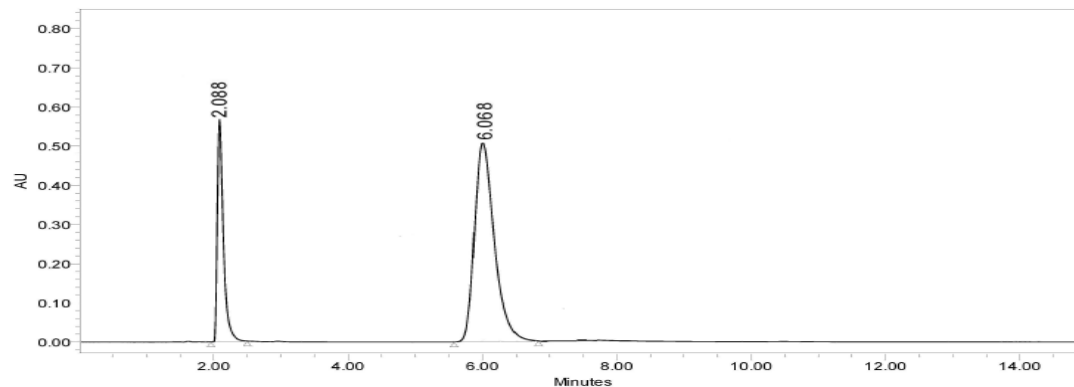


Fig-7.7: Optimized Chromatogram

Table7.7: Peak Results for Optimized Chromatogram

S. No.	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ranitidine	2.088	3425413	567933		1.0	5565.5
2	Metoclopramide	6.068	1629854	517733	2.5	1.1	5355.2

Observation:

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

Optimized Chromatogram (Sample)

Mobile phase : Methanol and water(65:35 v/v)
 Column : X- bridge column C18 (4.6 x 150mm, 5µm)
 Flow rate : 1 ml/min
 Wavelength : 260 nm
 Column temp : Ambient
 Injection Volume : 10 µl
 Run time : 14 minutes

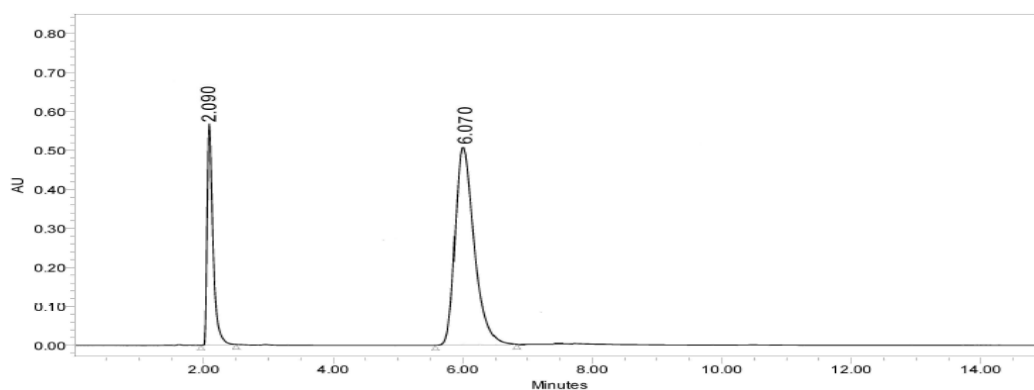


Figure-7.8: Optimized Chromatogram (Sample)

Table-7.8: Optimized Chromatogram (Sample)

S.No	Name	Retention time	Area (μV sec)	Height (μV)	USP resolution	USP tailing	USP plate count
1	Ranitidine	2.090	3468547	567933		1.0	5565.5
2	Metoclopramide	6.070	16289441	517733	2.5	1.1	5355.2

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.

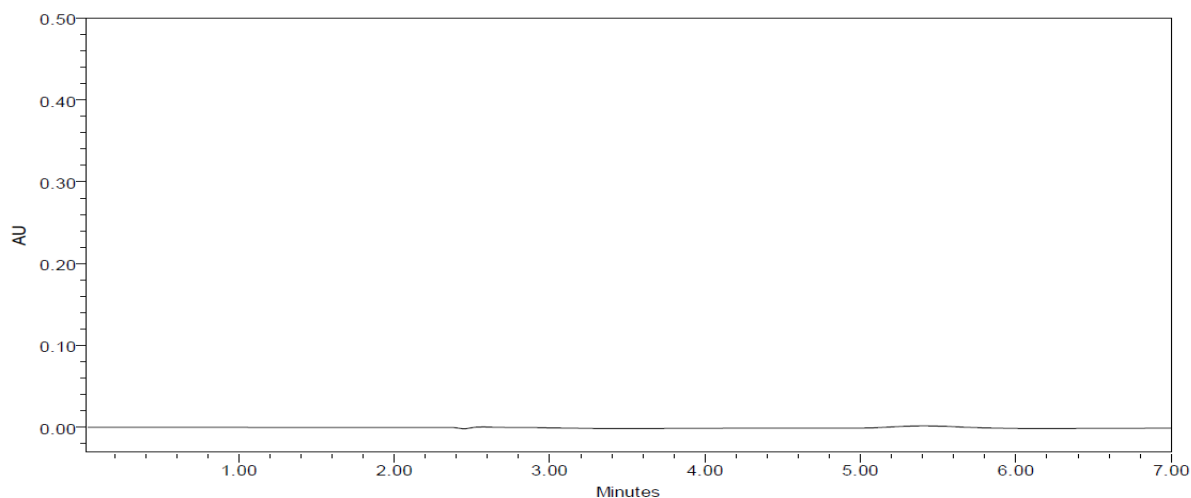
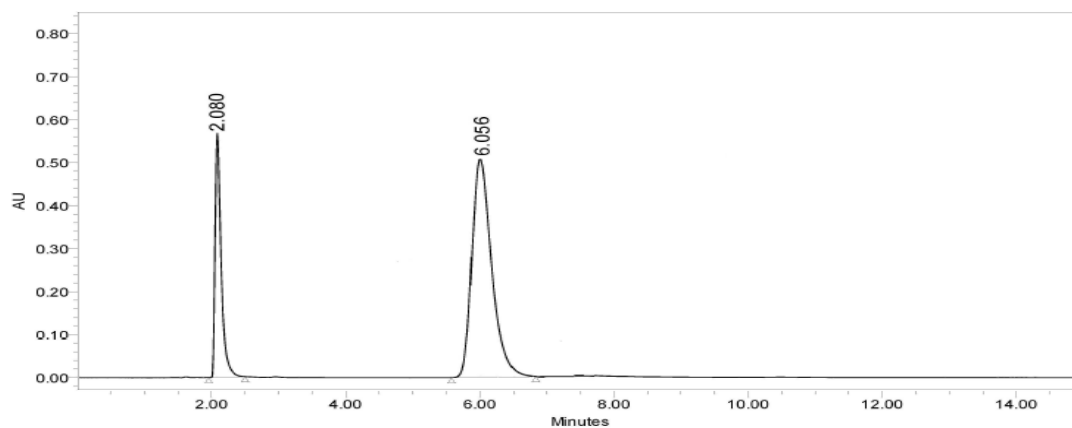
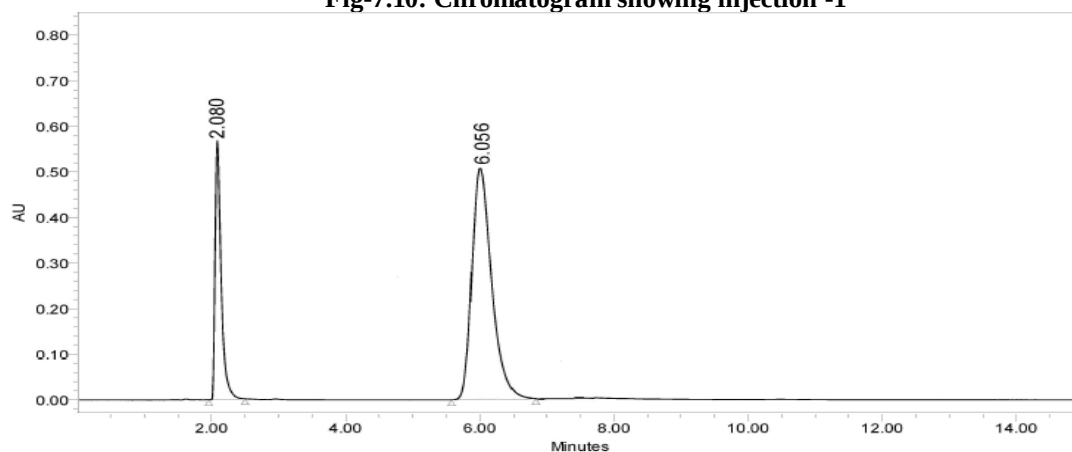
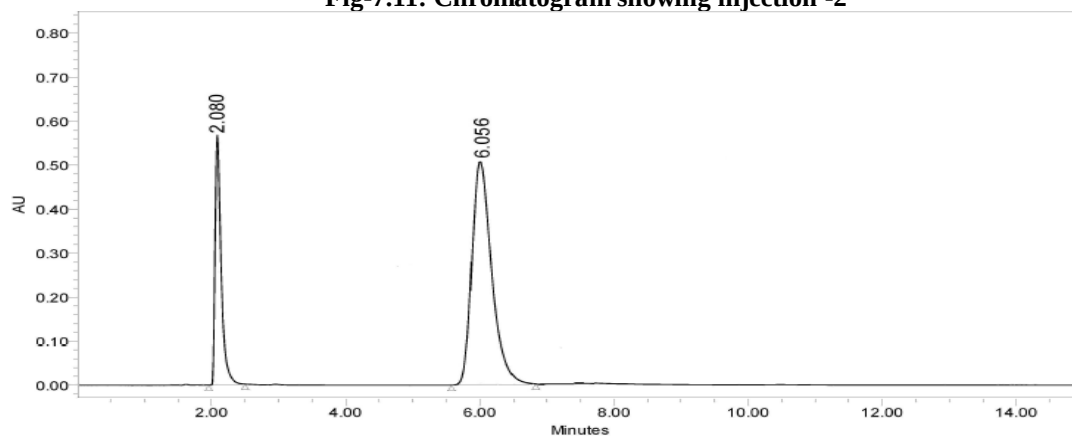
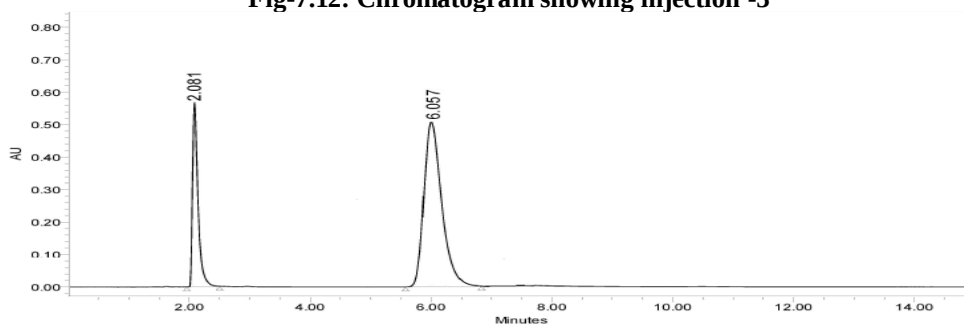
METHOD VALIDATION**Blank:**

Fig-7.9: Chromatogram showing blank (mobile phase preparation)

System Suitability:

**Fig-7.10: Chromatogram showing injection -1****Fig-7.11: Chromatogram showing injection -2****Fig-7.12: Chromatogram showing injection -3****Fig-7.13: Chromatogram showing injection -4**

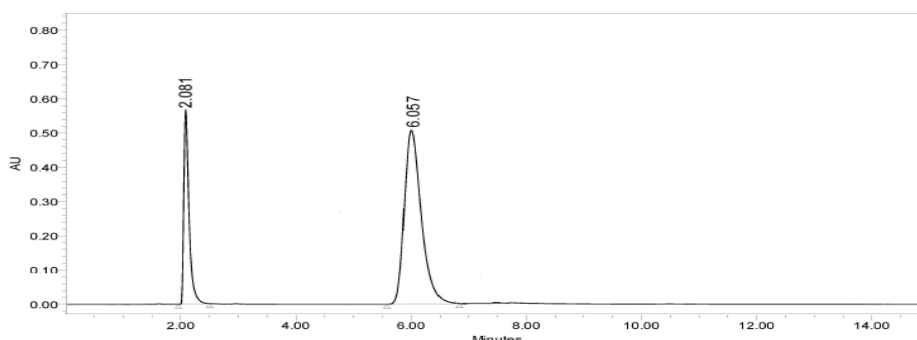


Fig7.14: Chromatogram showing injection -5

Table-7.9: Results of system suitability for Ranitidine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ranitidine	2.080	3569412	567917	5568.0	1.0
2	Ranitidine	2.080	3465125	517719	6359.2	1.1
3	Ranitidine	2.080	3598154	567933	5565.5	1.0
4	Ranitidine	2.081	3586491	517733	5355.2	1.1
5	Ranitidine	2.081	3582694	567917	6348.0	1.0
Mean			3560375			
Std. Dev			54225.61			
% RSD			1.523031			

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.

Table-7.10: Results of method precession for Metoclopramide:

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metoclopramide	2.080	3582264	567917	5568.0	1.0	2.5
2	Metoclopramide	2.080	3586491	517719	5359.2	1.1	2.5
3	Metoclopramide	2.080	3598154	567933	5565.5	1.0	2.5
4	Metoclopramide	2.081	3564125	517733	5355.2	1.1	2.5
5	Metoclopramide	2.081	3569412	562173	5568.0	1.0	2.5
Mean			3580089				
Std. Dev			13609.81				
% RSD			0.380153				

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

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