METHOD DEVELOPMENT ANDVALIDATION OF CLINDAMYCIN AND MICONAZOLE NITRATE IN SOFT GELATIN CAPSULES BY RP-HPLC

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In partial fulfillment of the requirements for the award of Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS

Submitted By

Reg No: 261330956

DEPARTMENT OF PHARMACEUTICAL ANALYSIS

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CERTIFICATE

This is to certify that the dissertation entitled **"METHOD DEVELOPMENT AND VALIDATION OF CLINDAMYCIN AND MICONAZOLE NITRATE IN SOFT GELATIN CAPSULES BY RP-HPLC"** submitted by **Naveenkumar** (Reg No: **261330956**) in partial fulfillment for the award of degree of Master of Pharmacy to the Tamilnadu Dr. M.G.R Medical University, Chennai is an independent bonafide work of the candidate carried out under my guidance in the Department of Pharmaceutical Analysis, Edayathangudy.G.S Pillay College of Pharmacy during the academic year 2015-2016.

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INDEX

1. INTRODUCTION [1,2,3,4]

Analytical chemistry may be defined as the "Science and art of determining the composition of materials in terms of the elements or compounds contained".

Analytical method is a specific application of a technique to solve an analytical problem. The use of instrumentation is an exciting and fascinating part of chemical analysis that interacts with all areas of chemistry and with many other areas of pure and applied science. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs, water and air. The manufacture of materials, whose composition must be known precisely, is to be monitored by analytical instruments.

Types of Instrumentation[5]

A) Spectrometric Techniques

- Atomic Spectrometry (Emission and Absorption)
- Electron Spin Resonance Spectroscopy
- Fluorescence and phosphorescence Spectrophotometry
- Infrared Spectrophotometry
- Nuclear Magnetic Resonance Spectroscopy
- Radiochemical Techniques including activation analysis
- Raman Spectroscopy
- Ultraviolet and visible Spectrophotometry
- X-Ray Spectroscopy

B) Chromatographic Techniques

- Gas Chromatography
- High performance Liquid Chromatography
- Thin Layer Chromatography

C) Miscellaneous Techniques

- Kinetic Techniques
- Mass Spectrometry
- Thermal Analysis

D) Hyphenated Techniques

- GC-MS (Gas Chromatography – Mass Spectrometry)
- ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry)
- GC-IR (Gas Chromatography – Infrared Spectroscopy)
- MS-MS (Mass Spectrometry – Mass Spectrometry)

HPLC

HPLC is a modern technique, it is a much more reliable and reproducible method for the standardization of both single and compound formulation. HPLC is a separation technique based on a stationary phase and a liquid mobile phase. Separations are achieved by partition, adsorption or ion exchange process, depending upon the size of stationary phase used.

HPLC is one of the most versatile instruments used in the field of pharmaceutical analysis. It provides the following features.

- High resolving power
- Speedy separation
- Continuous monitoring of the column effluent
- Accurate quantitative measurement
- Repetitive and reproducible analysis using the same column
- Automation of the analytical procedure and data handling.

Modes in HPLC

Quantitative methods

In all HPLC analysis, we are interested either in qualitative or quantitative determination of the different components present in the sample.

Qualitative analysis (Identification)

Initial identification of different components of the sample should be made by comparing retention time (RT) of different analytes with that of standard. Since, many substances can have similar RT values, use of different chromatographic parameters (different MP, different column, flow rate) to confirm the identity of the compound.

TYPES OF HPLC TECHNIQUES

Based on modes of chromatography:

- Normal phase chromatography
- Reverse phase chromatography

Based on principle of separation:

- Adsorption chromatography
- Ion exchange chromatography
- Size exclusion chromatography
- Affinity chromatography
- Chiral phase chromatography

Base on elution technique:

- Isocratic separation
- Gradient separation

Based on the scale of operation:

- Analytical HPLC
- Preparative HPLC

NORMAL PHASE CHROMATOGRAPHY.

- **Mechanism:** Retention by interaction of the polar surface of stationary phase with polar parts of the sample molecules.

- **Stationary Phase:** It is a bonded siloxane with polar functional groups like SiO_2, Al_2O_3, $-NH_2$, -CN, $-NO_2$, - Diol.

- **Mobile Phase:** Nonpolar solvents like heptane, hexane, cyclohexane, chloroform, ethyl ether, and dioxane.
- **Application:** Separation of nonionic, nonpolar to medium and polar substances.
- **Sample Elution Order:** Least polar components are eluted first.

REVERSE PHASE CHROMATOGRAPHY

- **Mechanism:** Retention by interaction of the nonpolar hydrocarbon chain of stationary phase with nonpolar parts of sample molecules.

- **Stationary Phase:** It is bonded siloxane with nonpolar functional groups like n-octadecyl (C_{18}) or n-octyl (C_8), ethyl, phenyl, $-(CH_2)$ n-diol, $-(CH_2)$n-CN.

- **Mobile Phase:** Polar solvents like methanol, acetonitrile, water or buffer (Sometimes with additives of THF or dioxane).
- **Application:** Separation of nonionic and ion forming nonpolar to medium polar substances.

- **Sample Elution Order:** Most polar components are eluted first.

PRINCIPLES OF SEPARATION

Adsorption chromatography employs high-surface area particles that absorb the solute molecules. Usually a polar solid such as a silica gel, alumina or porous glass beads and a non-polar mobile phase such as heptane, octane or chloroform are used in adsorption chromatography. In adsorption chromatography, adsorption process is described by competition model and solvent interaction model.

ION-EXCHANGE CHROMATOGRAPHY

Separation is based on the charge-bearing functional groups, anion exchange for sample negative ion, or cation exchange - for sample positive ion. Gradient elution by pH is common.

SIZE EXCLUSION CHROMATOGRAPHY

Also known as gel permeation or filtration, separation is based on the molecular size or hydrodynamic volume of the components. Molecules that are too large for the pores of the porous packing material on the column elute first, small molecules that enter the pores elute last, and the elution rates of the rest depend on their relative sizes.

AFFINITY/ ION-PAIR CHROMATOGRAPHY

Separation is based on a chemical interaction specific to the target species. The more popular reversed phase mode uses a buffer and an added counter-ion of opposite charge to the sample with separation being influenced by pH, ionic strength, temperature, concentration of and type of organic co-solvent(s). Affinity chromatography, common for macromolecules, employs a ligand (biologically active molecule bonded covalently to the solid matrix) which interacts with its homologous antigen (analyte) as a reversible complex that can be eluted by changing buffer conditions.

CHIRAL CHROMATOGRAPHY:

Separation of the enantiomers can be achieved on chiral stationary phases by formation of diastereomers via derivatizing agents or mobile phase additives on a chiral stationary phase. When used as an impurity test method, the sensitivity is enhanced if the enantiomeric impurity elutes before the enantiomeric drug.

ISOCRATIC SEPARATION:

In this technique, the same mobile phase combination is used throughout the process of separation. The same polarity or elution strength is maintained the process.

GRADIENT SEPARATION:

In this technique, a mobile phase combination of lower polarity or elution strength is used followed by gradually increasing the polarity or elution strength.

ANALYTICAL HPLC:

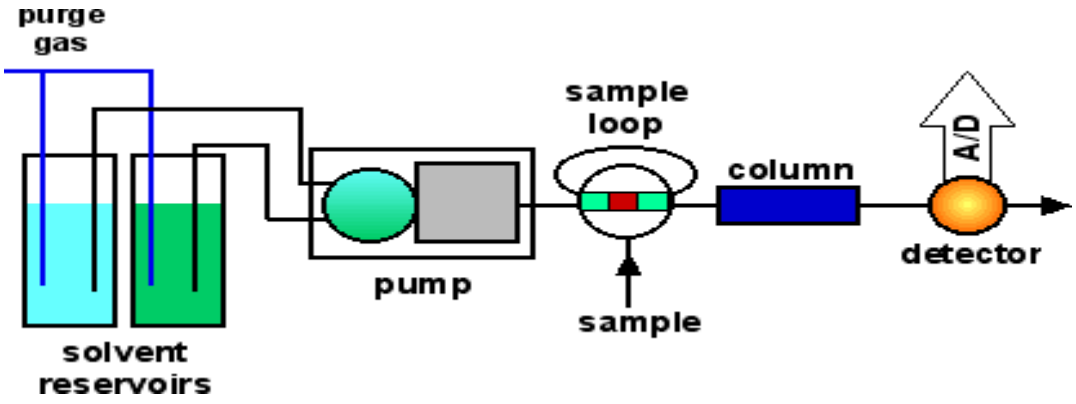
In this only analysis of the samples are done. Recovery of the samples for reusing is normally not done, since the samples used are very low.

PREPARATIVE HPLC:

Where analysis of the individual fractions of pure compounds can be collected using fraction collector. The collected samples are reused.

INSTRUMENTATION[7]

Typical diagram of HPLC[8]



The individual components HPLC and their working functions are described.[8]

SOLVENT DELIVERY SYSTEM

The most important component of HPLC in solvent delivery system is **the pump**, because its performance directly effects the retention time, reproducibility and detector sensitivity. Among the several solvent delivery systems,

Direct gas pressure, Pneumatic intensifier, Reciprocating pump with twin or triple pistons is widely used, as this system gives less baseline noise, good flow rate reproducibility etc.

MOBILE PHASE

The following points should also be considered when choosing a mobile phase:

1. It is essential to establish that the drug is stable in the mobile phase for at least the duration of the analysis.

2. Excessive salt concentrations should be avoided. High salt concentrations can result in precipitation, which can damage HPLC equipment.

3. The mobile phase should have a pH 2.5 and pH 7.0 to maximize the lifetime of the column.

4. Reduce cost and toxicity of the mobile phase by using methanol instead of acetonitrile when possible.

5. Minimize the absorbance of buffer. Since trifluoroacetic acid, acetic acid or formic acid absorb at shorter wavelengths, they may prevent detection of products without chromophores above 220 nm. Carboxylic acid modifiers can be frequently replaced by phosphoric acid, which does not absorb above 200 nm.

6. Use volatile mobile phases when possible, to facilitate collection of products and LC-MS analysis. Volatile mobile phases include ammonium acetate, ammonium phosphate, formic acid, acetic acid, and trifluoroacetic acid. Some caution is needed as these buffers absorb below 220 nm.

SOLVENT DEGASSING SYSTEM

The constituents of the mobile phase should be degassed and filtered before use. Several methods are employed to remove the dissolved gases in the mobile phase. They include

Heating and stirring, Vacuum degassing with an aspirator, Filtration through 0.45 filter, Vacuum degassing with an air-soluble membrane, Helium purging , purging or combination of these methods. HPLC systems are also provided an online degassing system, which continuously removes the dissolved gases from the mobile phase.

GRADIENT ELUTION DEVICES

HPLC columns may be run isocratically, i.e., with constant eluent or they may be run in the gradient elution mode in which the mobile phase composition varies during run. Gradient elution over comes the problem of dealing with a complex mixture of solutes.

COLUMNS

The heart of the system is the column. The choice of common packing material and mobile phases depends on the physical properties of the drug. The flow chart in Table.1 can assist one in determining which columns to examine. Many different reverse phase columns will provide excellent specificity for any particular separation. It is therefore best to routinely attempt separations with a standard C_8 or C_{18} column and determine if it provides good separations. If this column does not provide good separation

or the mobile phase is unsatisfactory, alternate methods or columns should be explored. Reverse phase columns differ by the carbon chain

COLUMN PACKING:

The packing used in modern HPLC consist of small, rigid particles having a narrow particle size distribution. There are three main type of column packing in HPLC.

- **Porous, Polymeric Beads**

  Porous, polymeric beds based on styrene divinyl benzene co-polymers used for ion exchange and size exclusion chromatography. For analytical purpose these have now been replaced by silica based packing which are more efficient and more stable.

- **Porous Layer Beds**

  This consisting of a thin shell (1-3 μm) of silica or modified silica on a spherical inert core (e.g. glass). After the development of totally porous micro particulate packing, these have not been used in HPLC.

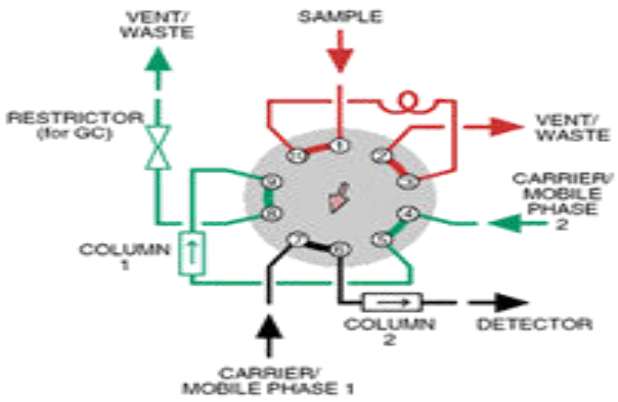
- **Totally Porous Silica Particles (dia. <10 µm)**

Particles of diameter >20µm are usually dry packed. While particles of diameter <20µm are slurry packed in which particles are suspended on a suitable solvent and the slurry is driven into the column under pressure.

SAMPLE INTRODUCTION SYSTEM

Two means for analyte introduction on the column are injection into a

Flowing stream, Stopflowinjection, Rheodyne injector. These techniques can be used with a syringe or an injection valve. Automatic injector is a microprocessor-controlled version of the manual universal injector. Usually, up to 100 samples can be loaded in to the auto injector tray. The system parameters such as flow rates, gradient, run time, volume to be injected, etc. are chosen, stored in memory and sequentially executed on consecutive injections.



INJECTORS

Sample introduction can be accomplished in various ways. The simplest method is to use an injection valve. In more sophisticated LC systems, automatic sampling devices are incorporated where sample introduction is done with the help of autosamplers and microprocessors.

In liquid chromatography, liquid samples may be injected directly and solid samples need only be dissolved in an appropriate solvent. The solvent need not be the mobile phase, but frequently it is judiciously chosen to avoid detector interference, column/component interference, and loss in efficiency.

TEMPERATURE

Room temperature is the first choice. Elevated temperatures are some times used to reduce column pressure or enhance selectivity. Typically, temperatures in excess of 60^0C are not used.

LIQUID CHROMATOGRAPHIC DETECTORS

UV/Visible Detector

A 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 (RI) Detector

Offers high sensitivity, stability and reproducibility, making 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.

Evaporative Light Scattering (ELS) Detector

A compact detector that controls temperatures in both the nebulization and evaporation stages, maintaining low-dispersion characteristics for dependable HPLC/ELS results.

Electrochemical Detector

Provides sensitivity for a variety of applications needs. It is configurable with a variety of flow cells, variable volumes, reference electrodes, working electrodes, and working electrode.

Conductivity Detector

Features a multi-electrode flow cell that offers exceptional sensitivity and stability for single-column or suppressor-based ion chromatography.

ANALYTICAL METHOD VALIDATION[9,10,11,12]

Validation is a basic requirement to ensure quality and reliability of the results for all analytical applications.

Validation is defined as follows by different agencies:

Method validation can be defined as per ICH,

"Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics

European Committee (EC)

Action of providing in accordance with the principles of good manufacturing practice that any procedure, process, equipment, material, activity or system actually

leads to the expected results. In brief validation is a key process for effective Quality Assurance.

Food and Drug administration (FDA)

Provides a high degree of assurance that specific process will consistently produce a product meeting its predetermined specification and quality attributes.

World Health Organization (WHO)

Action of providing that any procedure, process, equipment, material, activity, or system actually leads to the expected results.

SPECIFICITY/SELECTIVITY

The terms selectivity and specificity are often used interchangeably. According to ICH, the term specific generally refers to a method that produces a response for a single analyte only while the term selective refers to a method which provides responses for a number of chemical entities that may or may not be distinguished from each other. If the response is distinguished from all other responses, the method is said to be selective.

LINEARITY

Linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional the concentration (amount) of analyte in the sample. A linear relationship should be evaluated across the range of the analytical procedure.

RANGE

Range is the interval between the upper and lower concentration of the analyte in the sample for which it has a suitable level of precision, accuracy and linearity.

Range is derived from linearity studies in the method validation procedure. Some parameters with their ranges are shown in the table below,

Parameter	Range
Assay (Drug substance and finished product) Content uniformity Dissolution testing	80 – 120 % 70 – 130 % ±20 %

ACCURACY

Accuracy is the measure of the closeness of the experimental value is to the true value. Accuracy should be established across the specified range of the analytical procedure.

A. Assay

1.1 Drug Substance

Several methods of determining accuracy are available:

a) Application of an analytical procedure to an analyte of known purity (e.g. reference material);

b) Comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined.

c) Accuracy may be inferred once precision, linearity and specificity have been established.

Drug Product

Several methods for determining accuracy are available:

a) Application of the analytical procedure to synthetic mixtures of the drug product components to which known quantities of the drug substance to be analysed have been added.

b) In cases where it is impossible to obtain samples of all drug product components, it may be acceptable either to add known quantities of the analyte to the drug product or to compare the results obtained from a second, well characterized procedure, the accuracy of which is stated and/or defined.

c) Accuracy may be inferred once precision, linearity and specificity have been established.

Impurities (Quantitation)

Accuracy should be assessed on samples (drug substance/drug product) spiked with known amounts of impurities. In cases where it is impossible to obtain samples of certain impurities and/or degradation products

PRECISION:

Precision is the measure of how close the data values are to each other for a series of measurements under the same analytical conditions obtained from multiple sampling of the same homogeneous sample. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

A. Repeatability

1. Injection Repeatability

The sensitivity or precision as measured by multiple injections of a homogeneous sample (prepared solution) indicates the performance of the HPLC instrument under the chromatographic conditions and day tested. The information is provided as part of the validation data and as a system suitability test. The specification, as the coefficient of variation in % or relative standard deviation (RSD), set here will determine the variation limit of the analysis. The tighter the value, the more precise or sensitive to variation one can expect the results. This assumes that the chromatograph does not malfunction after the system suitability testing has been performed. The set of four duplicate samples were injected sequentially. Variations in peak area and drift of retention times are noted.

2. Analysis Repeatability

Determination, expressed as the RSD, consists of multiple measurements of a sample by the same analyst under the same analytical conditions. For practical purpose, it is often combined with accuracy and carried out as a single study.

B. Intermediate precision

Intermediate precision was previously known as part of ruggedness. The attribute evaluates the reliability of the method in a different environment other than that used during development of the method. The objective is to ensure that the method will provide the same results when similar samples are analyzed once the method development phase is over. Depending on time and resources, the method can be tested on multiple days, analysts, instruments, etc.

C. Reproducibility

As defined by ICH, reproducibility expresses the precision between laboratories as in collaborative studies. Multiple laboratories are desirable but not always attainable because of the size of the firm

LIMIT OF DETECTION

These limits are normally applied to related substances in the drug substance or drug product. Specifications on these limits should be provided by the regulatory authorities.

A. Based on Visual Evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods. The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

B. Based on Signal-to-Noise

This approach can only be applied to analytical procedures which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit.

C. Based on the Standard Deviation of the Response and the Slope

The detection limit (DL) may be expressed as:

$$DL = \frac{3.3\ \sigma}{S}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of s may be carried out in a variety of ways, for example:

1. Based on the Standard Deviation of the Blank

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

2. Based on the Calibration Curve:

A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

Recommendations:

The detection limit and the method used for determining the detection limit should be presented. If DL is determined based on visual evaluation or based on signal to noise ratio, the presentation of the relevant chromatograms is considered acceptable for justification.

In cases where an estimated value for the detection limit is obtained by calculation or extrapolation, this estimate may subsequently be validated by the independent analysis of a suitable number of samples known to be near or prepared at the detection limit.

LIMIT OF QUANTIFICATION

Limit of quantification is the lowest concentration of analyte in a sample which can be quantitatively determined with acceptable precision and accuracy under the stated experimental conditions. Several approaches for determining the quantification limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

A. Based on Visual Evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods. The quantification limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

B. Based on Signal-to-Noise Approach

This approach can only be applied to analytical procedures that exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1.

C. Based on the Standard Deviation of the Response and the Slope

The quantification limit (QL) may be expressed as:

$$QL = \frac{10\,\sigma}{S}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of s may be carried out in a variety of ways for example:

1. Based on Standard Deviation of the Blank

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

2. Based on the Calibration Curve

A specific calibration curve should be studied using samples, containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

ROBUSTNESS

ICH defines robustness as a measure of the method's capability to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage

Examples of typical variations are:

- Stability of analytical solutions
- Extraction time In the case of liquid chromatography, examples of typical variations are
- Influence of variations of pH in a mobile phase
- Influence of variations in mobile phase composition
- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate. In the case of gas-chromatography, examples of typical variations are
- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate.

SYSTEM SUITABILITY PARAMETERS :

System suitability tests are most often applied to analytical instrumentation. They are designed to evaluate the components of the analytical system in order to show that the performance of the system meets the standard required by the method. After the method has been validated an overall system suitability tests should be routinely run to determine, if the operating system is performing properly.

Relative retention (selectivity):

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

Where,

α = Relative retention.

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

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

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

Theoretical plates:

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

Where, n = Theoretical plates.

t = Retention time of the component.

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

Capacity factor:

$K^1 = (t_2 / t_a) – 1$

Where K^1 = Capacity factor.

Resolution:

$R = 2\ (t_2 - t_1) / (w_2 + w_1)$

Where R = Resolution between a peak of interest (peak 2) and the peak preceding it (Peak 1).

W_2 = Width of the base of component peak 2.

W_1 = Width of the base of component peak 1.

Peak asymmetry:

$T = W_{0.05} / 2f$

Where 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.

Plates per meter:

$N = n / L$

Where N = Plates per meter.

L = Column length, in meters.

HETP = L / n

Linear fit:

A linear calibration fit determines the best line (linear regression) for a series of calibration points. A minimum of two calibration points is required to determine a linear fit. The equation for calibrating the uncorrected amount is:

$$[Y = a X + b]$$

Where, Y = Component area or height.

a = Slop of the calibration line.

X = Uncorrected amount.

b = Y- axis intercept of the calibration line.

2. LITERATURE REVIEW:

1. HPLC and chemometric methods for the simultaneous determination of miconazole nitrate and nystatin. Heneedak et al., 2012.

High-performance liquid chromatography (HPLC) and chemometric methods were applied to the simultaneous determination of the two nonsteroidal antifungal drugs, miconazole (MIC) and nystatin (NYS). The applied chemometric techniques are multivariate methods including classical least squares, principal component regression and partial least squares methods. The ultraviolet (UV) absorption spectra of the standard solutions of the training and validation sets in methanol are recorded in the range of 280-320 nm at 0.2-nm intervals. The HPLC method depends on reversed-phase separation using a C18 column. The mobile phase consists of a mixture of methanol-acetonitrile-ammonium acetate buffer (pH 6; 50 mM) (60:30:10 v/v/v). The UV detector was set at 230 nm. The developed methods were validated and successfully applied to the simultaneous determination of MIC and NYS in their tablets. The assay results obtained using the chemometric methods were statistically compared to those of the HPLC method and good agreement was observed.

2. Development and validation of a simple stability-indicating high performance liquid chromatographic method for the determination of miconazole nitrate in bulk and cream formulations María et al ., 2008.

A simple and stability-indicating high performance liquid chromatographic method was developed and validated for the determination of miconazole nitrate in bulk and cream preparations. The extraction step for cream samples consisted in a warming,

cooling and centrifugation procedure that assures the elimination of the lipophilic matrix component, in order to avoid further precipitation in the chromatographic system. Separation was achieved on a ZORBAX Eclipse XDB – C18 (4.6 mm × 150 mm, 5 μm particle size) column, using a mobile phase consisting of water, methanol and acetonitrile, in a flow and solvent gradient elution for 15 min. The column was maintained at 25 °C and 10 μL of solutions were injected. UV detection was performed at 232 nm, although employment of a diode array detector allowed selectivity confirmation by peak purity evaluation. The method was validated reaching satisfactory results for selectivity, precision and accuracy. Degradation products in naturally aged samples could be simultaneously evaluated, without interferences in the quantitative analysis.

3.The Determination of Miconazole and its Related Production Impurities Together with Basic Solution Stability Studies Using a Sub 2 mm Chromatographic Column. N. O'Connor1 et al., 2012

A selective and sensitive method for the analysis of Miconazole and its associated impurities is developed. The separation is carried out using a Thermo Scientific Hypersil Gold C18 Column (50 mm x 4.6 mm i.d., 1.9 mm particle size) with a mobile phase of acetonitrile–methanol–ammonium acetate (1.5 w/v) (30:32:38 v/v) at a flow rate of 2.5 mL/min and UV detection at 235 nm. The method is validated according to ICH guidelines with respect to precision, accuracy, linearity, specificity, robustness, and limits of detection and quantification. All parameters examined are found to be well within the stated guidelines. Naturally aged samples are also tested to determine sample stability. A profile of sample andimpurity breakdown was presented. The analysis time was more than halved from just under 20 min (the current European Pharmacopeia Method) to under 8 min (developed method) and the method is applicable for assay and related substance determination..

4. Simultaneous determination of metronidazole and miconazole in pharmaceutical dosage forms by RP-HPLC. Akay et al 2002.

A reversed-phase high performance liquid chromatography (RP-HPLC) method with UV detection is described for the simultaneous determination of metronidazole and miconazole in pharmaceutical dosage forms. Chromatography was carried out on a C18 reversed-phase column, using a mixture of methanol–water (40+60, v/v) as a mobile phase, at a flow rate of 1.0 ml min^{-1}. Sulfamethoxazole was used as an internal standard and detection was performed using a diode array detector at 254 nm. The method produced linear responses in the concentration ranges 10–70 and 1–20 μg ml^{-1} with detection limits 0.33 and 0.27 μg ml^{-1} for metronidazole and micanozole, respectively. This procedure was found to be convenient and reproducible for analysis of these drugs in ovule dosage forms.

5. Development and validation of a gradient HPLC method for the determination of clindamycin and related compounds in a novel tablet formulation. *Daniel J. Platzer et al.,* 2006.

A gradient reversed-phase HPLC method was developed and validated for potency, content uniformity, and impurity determinations for a novel tablet formulation containing clindamycin. The assay utilized UV detection at 214 nm and a Waters Xterra RP_{18} column (4.6 mm × 100 mm, 3.5 μm). The mobile phases were comprised of pH 10.5, 10 mM carbonate buffer and acetonitrile. Validation experiments were performed to demonstrate specificity, linearity, accuracy (i.e., average recovery from the formulation), precision (i.e., repeatability), limit of quantitation (LOQ), and robustness (i.e., sample solution stability and buffer pH effects on specificity). The assay was shown to be specific for clindamycin, several impurities, and triethyl citrate, a retained excipient that was present in the dosage form. The assay was proved linear (concentration versus peak area) for clindamycin

and several select impurities over the ranges of 70–130% and 0.1–5%, respectively. UV relative response factors were determined for the impurities from the linearity data. The accuracy of clindamycin at the targeted assay concentration was 99.2% ($n = 3$; precision = 0.12%, R.S.D.); accuracy for lincomycin, a structurally related impurity, was 97.4% ($n = 3$; precision = 3.5%, R.S.D.) at 0.1% of the targeted assay concentration. By demonstrating an acceptable degree of precision for lincomycin at this level, the LOQ was shown to be no higher than 0.1%. The chromatography was virtually unaffected over a mobile phase buffer pH range spanning 0.4 pH units. Sample solutions were stable for 72 h under ambient conditions.

6. A New HPLC/UV method for the determination of clindamycin in dog blood serum. *Batzias et al* ., 2004.

A New HPLC method for the quantitative determination of clindamycin in dog blood serum at levels down to 80 ng/ml has been developed. Samples were deproteinised with acetonitrile and clindamycin was extracted with dichloromethane. Chromatographic analysis was carried out on a C_{18} reversed-phase analytical column in the presence of tetra-*n*-butylammonium hydrogen sulfate (TBA), as an ion-pairing agent. UV detector wavelength was set at 195 nm. The assay was validated for a concentration range from 80 to 6000 ng/ml serum. Good linearity was observed in the entire concentration range. The limit of quantification (LOQ) was 80 ng/ml and the limit of detection (LOD) was 60 ng/ml. Regression of accuracy data yielded an overall mean recovery value (±S.E.M.) of 93.98±0.42%, while precision data revealed coefficient of variation (CV (%)) values lower than 4.41%. The method was successfully applied to determine drug concentrations in serum samples from dogs that had been orally administered clindamycin hydrochloride.

7. Simple method for the assay of clindamycin in human plasma by reversed-phase high-performance liquid chromatography with UV detector. Cho SH et al. , 2005.

A rapid and simple high-performance liquid chromatography (HPLC) method was developed and validated for the quantification of clindamycin in human plasma. After precipitation with 50% trichloroacetic acid (TCA) containing the internal standard, propranolol, the analysis of the clindamycin level in the plasma samples was carried out using a reverse-phase cyano (CN) column with ultraviolet detection (204 nm). The chromatographic separation was accomplished with an isocratic mobile phase consisting of acetonitrile-distilled water-7.6 mm tetramethylammonium chloride (TMA) (60:40:0.075, v/v/v), adjusted to pH 3.2. The proposed method was specific and sensitive with a lower limit of quantitation (LLOQ) of 0.2 microg/mL. This HPLC method was validated by examining the precision and accuracy for inter- and intraday analysis in the concentration range 0.2-20.0 microg/mL. The relative standard deviations (RSD) in the inter- and intraday validation were 6.1-14.9 and 6.0-16.1%, respectively. In the stability test, clindamycin was found to be stable in human plasma during the storage and assay procedure. The present HPLC method was applied to the analysis of samples taken up to 12 h after a single oral administration of clindamycin in healthy volunteers.

8.Development and validation of hplc method for simultaneous estimation of clindamycin phosphate, clotrimazole and tinidazole in pharmaceutical dosage form *Darji R B, Patel B H.,* 2012.

A simple, rapid, precise, stable and accurate liquid chromatographic method (HPLC) was developed for the simultaneous estimation of Clindamycin phosphate (CLI), Clotrimazole (CLO) and Tinidazole (TIN) in pharmaceutical dosage form. A Kromasil C18 column (150 MM ×4.6 MM, 5 µm) in isocratic mode using ph 2.5

±0.01 phosphate buffer and methanol in the ratio of 40:60 as a mobile phase was used. The flow rate was maintained at 1 mL/min. The detection was carried out at 210 nm. The column temperature was maintained at 40 oC. The retention time was found to be 2.7 min, 3.9 min and 8.5 min TIN, CLI and CLO respectively. The method was validated for linearity, accuracy, precision and robustness. The assay was linear over the range of 75-350 µg/ml. The average recovery of TIN, CLI and CLO was found to be 99.87±0.64 %, 100.61±1.02 % and 100.03±0.84 % respectively. The percentage relative standard deviation (%RSD) was found to be less than 2 % in precision study for each drug. The proposed method was successfully applied for the quantitative determination of CLI, CLO and TIN in pharmaceutical dosage form.

9. LC Determination of Clindamycin Phosphate from Chitosan Microspheres, Muge et al., 2010.

A simple, rapid and precise reverse phase LC method was adopted, modified and validated for the determination of clindamycin phosphate from chitosan microspheres prepared by spray drying method. Separation was performed using ACE5 C18 reversed phase column (150 mm × 4.6 mm, 5 µm) with acetonitrile:phosphate buffer at pH 2.5 (25:75 *v/v*) as mobile phase. The limit of detection was 46.43×10^{-3} µg mL^{-1}, with UV detection at 210 nm. No interference from chitosan and other excipients was observed. Therefore an incorporation efficiency of microspheres could be determined accurately and specifically.

10. Development and validation of a new HPLC analytical method for the quality control of clindamycin capsules. Tamaddon et al., 2012.

A simple and rapid isocratic reversed-phase high performance liquid chromatography (HPLC) method was developed, validated and applied for quantitation of clindamycin

hydrochloride in capsules. The chromatographic method employed on a Nucleodur© CN-RP column (250mm×4.6mm i.d., 5mm particle size) at ambient temperature. The mobile phases were comprised of a mixture of water and acetonitrile containing tetramethyl ammonium(pH 4.2) (60:40 v/v) at a flow rate of 1 ml/min. The UV detection was made at 204 nm. Propyl paraben was used as the internal standard. The average retention times for internal standard and clindamycin were 5.1 and 7.8 min respectively. The calibration curve was linear (r ¡Ý 0.998) over the concentration ranges of 2-9 µg/ml of clindamycin with detection limit of 0.3 µg/ml. Intra- and inter-day relative standard deviations were less than 2%. No chromatographic interferences from the capsule excipients were found. Results showed, the reported HPLC method for clindamycin provides several advantages of simplicity, high specificity, accuracy and short run-cycle time. This proposed method was successfully used in analyzing the drug in dissolution media and capsule formulations. The method may be used for the routine quality control analysis of clindamycin pure drug and its pharmaceutical preparations and even under certain circumstances for the drug bio- analysis.

3. AIM & OBJECTIVE:

There were no methods have been reported in the literature for the estimation of my method. Hence we made an attempt to develop a Assay method by HPLC for the estimation of Clindamycin, and Miconazole nitrate in Clindamycin 100mg and Miconazole nitrate 200mg Capsules (finished product) to demonstrate that the analytical procedure is suitable for its intended purpose.

The report applies to analytical method validation of Clindamycin and Miconazole nitrate, in Clindamycin 100mg and Miconazole nitrate 200mg Capsules.

4. PLAN OF WORK

- To develop a new RP-HPLC assay method for the estimation of clindamycin and miconazole nitrate in capsule dosage form.
- And To validate the developed method.

5. DRUG PROFILE :

HISTORY

Name : **Clindamycin Phosphate**

Chemical Name:

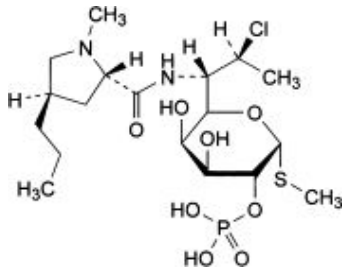
Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-propyl- L-pyrrolidinecarboxamido)-1-thio - L- threo- a- D -galacto-octopyranoside- 2-(dihydrogen phosphate)

Structure:

Molecular Formula: $C_{18}H_{34}ClN_2O_8PS$

Molecular Weight: 505.0

Molecular Structure:



CHARACTERS

A white or almost white powder, slightly hygroscopic, freely soluble in water, very slightly soluble in alcohol, practically insoluble in methylene chloride.

It shows polymorphism

Action and use:

Antibacterial.

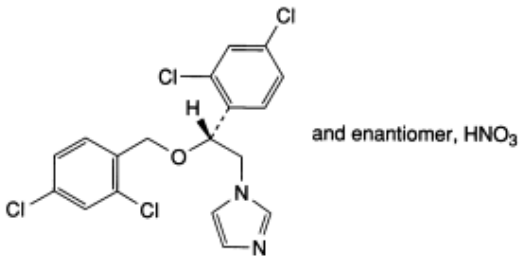
Name : **Miconazole Nitrate**

Chemical Name:

1-[(2*RS*)-2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole nitrate
Structure:

Molecular Formula: $C_{18}H_{14}Cl_4N_2O.HNO_3$

Molecular Weight: 479.10
Molecular Structure:



CHARACTERS:

A white or almost white powder, very slightly soluble in water, sparingly soluble in methanol, slightly soluble in alcohol.

Action and use: Antifungal, Antifungal

5. MATERIALS & METHODS

EXPERIMENTAL DETAILS

Equipment and Apparatus Used:

- Shimadzu LC 2010 - PDA detector.
- Chromatographic data Software: LC Solution.
- C_8 – 250 mm x 4.6 mm, 5µm
- Vacuum filter pump
- Heating mantle
- Ultrasonicator
- Waterbath
- Hot air oven
- Single pan balance (Metler Toledo)
- pH meter (elicos)
- refrigerator
- photostabilitychamber
- high pure water-sartorius(milliq water)

Reagents used:

a. potassium dihydrogen ortho phosphate
b. ortho phosphoric acid
c. Acetonitrile
d. Methanol
e. Water (HPLC).

METHOD DEVELOPMENT:

Principle:

Separation and quantification is based on the isocratic reverse phase chromatography with UV detection.

Reagents:

Buffer preparation:

Weigh about 1.36 gm of potassium dihydrogen ortho phosphate into 1000ml with water and Adjust pH 2.5 using ortho phosphoric acid.

Preparation of mobile Phase:

Mixture 40 volumes of buffer, 30 Volumes Acetonitrile and 30 Volumes of Methanol mix well and sonicate.

Preparation of diluent:

Mixture 40 volumes buffer, 20 Volumes Acetonitrile and 40 Volumes of Methanol mix well and sonicate.

Standard Preparation:

Weigh accurately 124 mg of Clindamycin phosphate WS and 200mg of Miconazole nitrate WS in a 100 ml volumetric flask, add 70 ml of diluent, shake well to dissolve and make up the volume to 100ml with diluent. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Sample Preparation:

Collect the Medicament, take one capsule fill weight in a 100 ml volumetric flask, and add 30 ml of diluent, shake well to dissolve and make up the volume to 100 ml with diluent. Mix well and Filter. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Chromatographic parameters:

Instrument	**HPLC with UV/PDA detector**
Column	C_8 – 250 mm x 4.6 mm, 5µm
Flow rate	1.5 ml/min.
Oven temperature	40°C±2°C
Sample temperature	Ambient
Wavelength	210 nm
Injection volume	20 µl
Run time	20 minutes

Procedure:

Inject the Diluent, standard and sample preparations as per the following sequence.

Solution Name	No of injections	Purpose
Diluent	1	Blank
Standard solution	6	System suitability &Quantification
Sample solution	1	Analysis

Record the chromatograms and measure the responses for the peak due to Clindamycin Phosphate and Miconazole nitrate in the standard and sample preparation.

Calculation:

For Clindamycin:

$$= \frac{T_A \times S_{WT} \times 10 \times 100 \times 100 \times S_P \times 0.812 \times \text{Avg.Fill wt.}}{S_A \times 100 \times 100 \times S_{WT} \times 10 \times 100 \times \text{Label claim}}$$

Where,

T_A – Area of Clindamycin in sample solution.

S_{WT} – Weight of Clindamycin WS taken in standard solution (mg).

S_P - % potency of Clindamycin Phosphate WS.

S_A – Average area of Clindamycin in standard solution.

T_{WT} – Weight of medicament taken in sample solution (mg).

Conversion Factor: Clindamycin Phosphate equivalent to Clindamycin = 0.812.

For Miconazole:

$$= \frac{T_A \times S_{WT} \times 10 \times 100 \times 100 \times S_P \times \text{Avg.Fill wt.}}{S_A \times 100 \times 100 \times S_{WT} \times 10 \times 100 \times \text{Label claim}}$$

Where,

T_A – Area of Miconazole in sample solution.

S_{WT} – Weight of Miconazole WS taken in standard solution (mg).

S_P - % Potency of Miconazole WS.

S_A – Average area of Miconazole in standard solution.

T_{WT} – Weight of medicament taken in sample solution (mg).

METHOD VALIDATION:

VALIDATION PARAMETERS:

THE FOLLOWING PARAMETERS SHALL BE CONSIDERED DURING ANALYTICAL METHOD VALIDATION

S.No	Parameter
1	System suitability
2	Specificity
3	Linearity and range
4	Accuracy
5	Precision System precision Intermediate precision
6	Ruggedness
7	Robustness
8	Filter Validation
9	Stability of Analytical solution (Standard and sample solution)

SYSTEM SUITABILITY:

Standard solution were prepared as per test method and injected into the HPLC System. The system suitability parameters were evaluated for , USP Plate count, USP tailing and relative standard deviation for Clindamycin Phosphate and Miconazole nitrate peak area from the chromatograms of Six replicate injections.

The Standard preparation, prepared by using Clindamycin Phosphate and Miconazole nitrate working standard as per test method was injected six times into the HPLC System.

Preparation of standard solution:

Weigh accurately 124 mg of Clindamycin phosphate WS and 200mg of Miconazole nitrate WS in a 100 ml volumetric flask, add 70 ml of diluent, shake well to dissolve and make up the volume to 100ml with diluent. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Inject the Diluent and standard solutions as per the following sequence:

Solution Name	No of injections	Purpose
Diluent	1	Blank
Standard solution	6	System suitability

Check the % RSD of areas, % RSD of RT, Theoretical plate count and tailing factor of Clindamycin and Miconazole peak.

Acceptance criteria:

System Suitability Parameters	**Acceptance criteria**
Tailing factor for Clindamycin and Miconazole peak from first standard injection.	**NMT 2.0**

Theoretical plate count for Clindamycin and Miconazole peak from first standard injection.	**NLT 2000**
The %RSD of RT for six injection of standard solution	**NMT 1.0**
The %RSD of Peak response for six injection of standard solution	**NMT 2.0**

SPECIFICITY:

"Specificity is the ability of the method to measure the analyte in the presence of matrix components. Demonstrate the specificity by identification of analytes, Blank and placebo interference and Peak purity of analyte."

Prepare the following solution for Specificity

Bank (Diluent)

Placebo Preparation: Weigh and transfer 1440 mg of the Placebo in to 100mL volumetric Flask and follow the final procedure of test preparation.

1.0 Prepare standard as per method.

2.0 Prepare test preparation as per method of analysis.

Summarize the results in the table given below.

Inject the Diluent, Placebo, Standard solution and sample solution as per the following sequence.

Solution Name	No of injections	Purpose
Diluent	1	To check the interference of blank and placebo peaks with the main analyte.
Placebo	1	
Standard solution	1	
Sample solution	1	

Acceptance Criteria:

1) There should not be any interference by blank, Placebo peaks with the main analyte peak.

2) The Purity angle index of sample peak and sample peak should not less than 0.99 (For LC solution software).

LINEARITY & RANGE:

Linearity:

The ability of the method to produce results is directly proportional to the concentration of the analyte in samples within a given range.

Range:

Is the interval between the upper and lower concentration of analyte for which has been demonstrated that the analytical method has a suitable level of precision, accurac and linearity".

Perform the linearity in the concentrations of 50.0%, 75.0%, 100.0%, 125.0%, and 150.0% of working concentration of Clindamycin and Miconazole nitrate.

Record the average area for each level and calculate slope, y- intercept & correlation coefficient.

Plot the graph of respective analyte peak concentration on X- axis and area response on Y-axis.

Standard Stock Solution for Linearity:

Weigh accurately 124 mg of Clindamycin phosphate WS and 200mg of Miconazole nitrate WS in a 100 ml volumetric flask, add 30 ml of diluent, shake well to dissolve and make up the volume to 100ml with diluent.

Preparation of Linearity Solutions:

1) **50.0% solution:**

Pipette 5ml standard stock solution to a 100.0 ml volumetric flask, make up volume with diluent.

2) **75.0% solution:**

Pipette 7.5ml standard stock solution to a 100.0 mL volumetric flask, make up volume with diluent.

3) **100.0% solution:**

Pipette 10.0ml standard stock solution to a 100.0 ml volumetric flask, make up volume with diluent.

4) **125.0% solution:**

Pipette 12.5ml standard stock solution to a 100.0 ml volumetric flask, make up volume with diluent.

5) 150.0% solution:

Pipette 15.0 ml standard stock solution to a 100.0 ml volumetric flask, make up volume with diluent.

Acceptance criteria:

Correlation coeff. (r^2) – Shall be NLT 0.999

Injection sequence:

Inject the solutions as per the following sequence

Solution Name	No of injections	Purpose
Diluent	1	To check the 1. Correlation coeff. (r^2) – 2. y- Intercept
50.0% solution	3	
75.0% solution:	3	
100.0% solution:	3	
125.0% solution:	3	
150.0% solution:	3	

Acceptance criteria:

System Suitability Parameters	**Acceptance criteria**
Correlation coeff. r^2	**NLT 0.999**

ACCURACY:

Accuracy is the closeness of the test results obtained by the method to the true value. Accuracy may often be expressed as percent recovery by the assay of known, added amounts of analyte. Accuracy is a measure of the exactness of analytical method.

Accuracy shall be assessed using '3' concentration (50.0%, 100.0%, and 150.0%).

The active can be added to placebo capsules at 50.0%, 100.0%, and 150.0% concentration and each subsequent mixture shall be assayed.At each concentration, the average result shall then be expressed as a percentage of the amount added, to determine the recovery at each level or concentration.

Preparation of Standard solution:

Weigh accurately 124 mg of Clindamycin phosphate WS and 200mg of Miconazole nitrate WS in a 100 ml volumetric flask, add 70 ml of diluent, shake well to dissolve and make up the volume to 100ml with diluent. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Test Solution: Prepare the below test solution in triplicate

50.0 % of test solution:

Weight accurately about 62mg Clindamycin Phosphate and 100mg Miconazole nitrate standard add about 1440mg of placebo and add 70 ml of diluent, shake well to dissolve and make up the volume 100ml with diluent, mix well. Filter. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

100.0 % of test solution:

Weight accurately about 124mg Clindamycin Phosphate and 200mg of Miconazole nitrate standard add about 1440mg of placebo and add 70 ml of diluent, shake well to dissolve and make up the volume 100ml with diluent, mix well. Filter. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

150.0 % of test solution:

Weight accurately above 186mg Clindamycin Phosphate and 300mg Miconazole nitrate standard add about 1440mg of placebo and add 70 ml of diluent, shake well to dissolve and make up the volume 100ml with diluent, mix well. Filter. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Injection sequence:

Solution Name	No of injections	Purpose
Diluent	1	To evaluate the accuracy of method by recovery
Standard solution	6	
50.0% of test solution-1	1	
50.0% of test solution-2	1	
50.0% of test solution-3	1	
100.0% of test solution-1	1	
100.0% of test solution-2	1	
100.0% of test solution-3	1	
150.0% of test solution-1	1	
150.0% of test solution-2	1	
150.0% of test solution-3	1	

Acceptance criteria:

System Suitability Parameters	Acceptance criteria

Tailing factor for Clindamycin and Miconazole peak from first standard injection.	**NMT 2.0**
Theoretical plate count for Clindamycin and Miconazole peak from first standard injection.	**NLT 2000**
The %RSD of RT for six injection of standard solution	**NMT 1.0**
The %RSD of Peak response for six injection of standard solution	**NMT 2.0**
% of recovery	**98.0 to 102.0**
% RSD of Recovery	**NMT 2.0**

PRECISION:

Determines closeness of agreement (expressed as Percentage Relative Standard deviation "% RSD") of the same homogenous sample under the prescribed conditions. The precision of the analytical method is determined by assaying 6 aliquots of homogeneous sample.

System Precision

System precision will be tested by injecting 6 replicate injections of Clindamycin phosphate and Miconazole nitrate working standard solution. Calculate % RSD of six peak area and RT.

Standard Preparation:

Weigh accurately 124 mg of Clindamycin phosphate WS and 200mg of Miconazole nitrate WS in a 100 ml volumetric flask, add 70 ml of diluent, shake well to dissolve and make up the volume to 100ml with diluent. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Injection sequence:

Inject the solutions as per the following sequence

Solution Name	No of injections	Purpose
Diluent	1	To perform system precision
Standard solution	6	

System Suitability Parameters	**Acceptance criteria**
Tailing factor for Clindamycin and Miconazole peak from first standard injection.	**NMT 2.0**
Theoretical plate count for Clindamycin and Miconazole peak from first standard injection.	**NLT 2000**

The % RSD of RT for six injection of standard solution	**NMT 1.0**
The % RSD of Peak response for six injection of standard solution	**NMT 2.0**

Method Precision

Method precision or reproducibility of solution will be demonstrated by analyzing the same Clindamycin and Miconazole nitrate Capsules batch above in 6 replicate samples.

Standard Preparation:

Weigh accurately 124 mg of Clindamycin phosphate WS and 200mg of Miconazole nitrate WS in a 100 ml volumetric flask, add 70 ml of diluent, shake well to dissolve and make up the volume to 100ml with diluent. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Sample Preparation:

Collect the Medicament, take one capsule fill weight in a 100 ml volumetric flask, and add 30 ml of diluent, shake well to dissolve and make up the volume to 100 ml with diluent. Mix well and Filter. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Injection sequence:

Inject the solutions as per the following sequence.

Solution Name	No of injections	Purpose
Diluent	1	Blank
Standard solution	6	System suitability &Quantification

Sample solution-1	1	To verify the precision
Sample solution-2	1	
Sample solution-3	1	
Sample solution-4	1	
Sample solution-5	1	
Sample solution-6	1	

Acceptance Criteria:

System Suitability Parameters	**Acceptance criteria**
Tailing factor for Clindamycin and Miconazole peak from first standard injection.	**NMT 2.0**
Theoretical plate count for Clindamycin and Miconazole peak from first standard injection.	**NLT 2000**
The %RSD of RT for six injection of standard solution	**NMT 1.0**
The %RSD of Peak response for six injection of standard solution	**NMT 2.0**
% of Assay	**NLT 90.0**
% RSD of assay of 6 replicate sample	**NMT 2.0**

Intermediate Precision:

Intermediate precision will be demonstrated by analysing the same Clindamycin and Miconazole nitrate Capsules batch as above in 6 replicate samples, in the same lab but by a different Analyst, using a different Instrument and on a different day.

Standard Preparation:

Weigh accurately 124 mg of Clindamycin phosphate WS and 200mg of Miconazole nitrate WS in a 100 ml volumetric flask, add 70 ml of diluent, shake well to dissolve and make up the volume to 100ml with diluent. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Sample Preparation:

Collect the Medicament, take one capsule fill weight in a 100 ml volumetric flask, and add 30 ml of diluent, shake well to dissolve and make up the volume to 100 ml with diluent. Mix well and Filter. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Injection sequence:

Inject the solutions as per the following sequence

Solution Name	No of injections	Purpose
Diluent	1	Blank
Standard solution	6	System suitability &Quantification
Sample solution-1	1	To verify the precision
Sample solution-2	1	
Sample solution-3	1	

Sample solution-4	1	
Sample solution-5	1	
Sample solution-6	1	

Acceptance criteria:

System Suitability Parameters	**Acceptance criteria**
Tailing factor for Clindamycin and Miconazole peak from first standard injection.	**NMT 2.0**
Theoretical plate count for Clindamycin and Miconazole peak from first standard injection.	**NLT 2000**
The %RSD of RT for six injection of standard solution	**NMT 1.0**
The %RSD of Peak response for six injection of standard solution	**NMT 2.0**
% of Assay	**NLT 90.0**
Confidence limits	**± 5.0%**
% RSD of assay of 6 replicate samples	**NMT 2.0**
% RSD of assay of 12 replicate samples by Analyst-1 & Analyst-2	

ROUGGEDNESS:

The Ruggedness has been demonstrated by injecting six samples prepared as per the test preparation given the method of analysis on two different days with different analysts and different system (minimum two different system).Calculate the mean, standard deviation, relative standard deviation and confidence limits for from six test preparations.

System to System variability

The Ruggedness has been demonstrated by injecting six samples prepared as per the test preparation given the method of analysis on two different days with different analysts and different system (minimum two different system).Calculate the mean, standard deviation, relative standard deviation and confidence limits for assay from six test preparations. Summarize the results in the table given below.

Standard Preparation:

Weigh accurately 124 mg of Clindamycin phosphate WS and 200mg of Miconazole nitrate WS in a 100 ml volumetric flask, add 70 ml of diluent, shake well to dissolve and make up the volume to 100ml with diluent. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Sample Preparation:

Collect the Medicament, take one capsule fill weight in a 100 ml volumetric flask, and add 30 ml of diluent, shake well to dissolve and make up the volume to 100 ml with diluent. Mix well and Filter. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluents

Injection sequence:

Inject the solutions as per the following sequence

Solution Name	No of injections	Purpose
Diluent	1	Blank
Standard solution	6	System suitability &Quantification
Sample solution-1	1	To verify the ruggedness
Sample solution-2	1	To verify the ruggedness

Acceptance criteria:

System Suitability Parameters	**Acceptance criteria**
Tailing factor for Clindamycin and Miconazole peak from first standard injection.	**NMT 2.0**
Theoretical plate count for Clindamycin and Miconazole peak from first standard injection.	**NLT 2000**
The %RSD of RT for six injection of standard solution	**NMT 1.0**
The %RSD of Peak response for six injection of standard solution	**NMT 2.0**
Confidence limits	**± 5.0%**

% of Assay 1. System-1 2. System-2	**NLT 90.0**
The % difference between the Assay obtained at system-1 and system-2	**NMT 2.0**

Column to Column variability:

The Ruggedness has been demonstrated injecting six samples prepared as per the test preparation given the method of analysis on two different days with different analysts and different column (minimum two different columns). Calculate the mean, standard deviation, relative standard deviation and confidence limits for assay from six test preparations. Summarize the results in the table given below.

Injection sequence:

Inject the solutions as per the following sequence

Solution Name	No of injections	Purpose
Diluent	1	Blank
Standard solution	6	System suitability &Quantification
Sample solution-1	1	To verify the ruggedness
Sample solution-2	1	To verify the ruggedness

Acceptance criteria:

System Suitability Parameters	Acceptance criteria
Tailing factor for Clindamycin and Miconazole peak from first standard injection.	**NMT 2.0**
Theoretical plate count for Clindamycin and Miconazole peak from first standard injection.	**NLT 2000**
The %RSD of RT for six injection of standard solution	**NMT 1.0**
The %RSD of Peak response for six injection of standard solution	**NMT 2.0**
Confidence limits	**± 5.0%**
% of Assay 1. Column-1 2. Column-2	**NLT 90.0**
The % difference between the Assay obtained at column-1 and Column-2	**NMT 2.0**

ROBUSTNESS:

The robustness of the analytical method for assay will be demonstrated by evaluation of small but deliberate variations in Clindamycin and Miconazole nitrate assay method chromatographic parameters. The % RSD of result data will be calculated.

The Robustness will be demonstrated for Clindamycin and Miconazole nitrate assay analytical method by changing chromatographic parameters. Parameters that influence the variations are ratio of mobile phase, flow rate, and wavelength

Effect of variation in mobile phase composition:

Effect of variation in Mobile phase composition (Methanol):

To demonstrate the robustness, check the system suitability parameters by injecting standard preparation, by using two mobile phases, one containing 95% of the method organic phase composition and other containing 105% of the method organic phase composition.

Preparation of mobile Phase: 100%

Mixture 30 volumes of Acetonitrile and 30 Volumes of Methanol and 40 volume of buffer mix well and sonicate.

Preparation of diluent:

Mixture 40 volumes buffer, 20 Volumes Acetonitrile and 40 Volumes of Methanol mix well and sonicate.

Preparation of mobile Phase: (95 % of methanol)

Mixture 30 volumes of Acetonitrile and 28.5 Volumes of Methanol and 40 volume of buffer mix well and sonicate.

Preparation of mobile Phase: (105 % of methanol)

Mixture 30 volumes of Acetonitrile and 31.5 Volumes of Methanol and 40 volume of buffer mix well and sonicate.

Standard Preparation:

Weigh accurately 124 mg of Clindamycin phosphate WS and 200mg of Miconazole nitrate WS in a 100 ml volumetric flask, add 70 ml of diluent, shake well to dissolve and make up the volume to 100ml with diluent. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Sample Preparation:

Collect the Medicament, take one capsule fill weight in a 100 ml volumetric flask, and add 30 ml of diluent, shake well to dissolve and make up the volume to 100 ml with diluent. Mix well and Filter. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Injection sequence:

Inject the solutions as per the following sequence

Solution Name	No of injections	Purpose
Diluent	1	Blank
Standard solution	6	System suitability &Quantification
Sample solution-1	1	To verify the robustness
Sample solution-2	1	To verify the robustness

***Sequence shall be repeated with all three different mobile phase compositions.**

Acceptance criteria:

System Suitability Parameters	Acceptance criteria
Tailing factor for Clindamycin Phosphate and Miconazole peak from first standard injection.	**NMT 2.0**
Theoretical plate count for Clindamycin Phosphate and Miconazole peak from first standard injection.	**NLT 2000**
The %RSD of RT for six injection of standard solution	**NMT 1.0**
The %RSD of Peak response for six injection of standard solution	**NMT 2.0**
% of Assay 1. 95% methanol Mobile phase 2. 100% methanol Mobile phase 3.105% methanol Mobile phase	**NLT 90.0**
The % difference between the Assay results obtained with three different mobile phase composition	**NMT 2.0**

Effect of variation in flow rate:

To demonstrate the robustness of test method, check the system suitability parameters by injecting standard preparations into the HPLC system with 1.3 ml/min, 1.5 ml/min and 1.7ml/min.

Standard Preparation:

Weigh accurately 124 mg of Clindamycin phosphate WS and 200mg of Miconazole nitrate WS in a 100 ml volumetric flask, add 70 ml of diluent, shake well to dissolve and make up the volume to 100ml with diluent. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Sample Preparation:

Collect the Medicament, take one capsule fill weight in a 100 ml volumetric flask, and add 30 ml of diluent, shake well to dissolve and make up the volume to 100 ml with diluent. Mix well and Filter. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Injection sequence:

Inject the solutions as per the following sequence

Solution Name	No of injections	Purpose
Diluent	1	Blank
Standard solution	6	System suitability &Quantification
Sample solution-1	1	To verify the robustness
Sample solution-2	1	To verify the robustness

***Sequence shall be repeated with all three different flow rates**

Acceptance Criteria:

System Suitability Parameters	**Acceptance criteria**
Tailing factor for Clindamycin Phosphate and Miconazole peak from first standard injection.	**NMT 2.0**

Theoretical plate count for Clindamycin Phosphate and Miconazole peak from first standard injection.	**NLT 2000**
The %RSD of RT for six injection of standard solution	**NMT 1.0**
The %RSD of Peak response for six injection of standard solution	**NMT 2.0**
% of Assay 1) 1.3 ml/minute flow rate. 2) 1.5ml/minute flow rate. 3) 1.7 ml/minute flow rate.	**NLT 90.0**
The % difference between the Assay results obtained with three different flow rates.	**NMT 2.0**

Effect of variation in Wavelength:

To demonstrate the robustness of **t**est method, check the system suitability parameters by injecting standard preparations into the HPLC system with 208nm 210nm and 212nm Wavelength. Calculate the mean, standard deviation and relative standard deviation for assay from two test preparations. Summarize the results in the table given below.

Standard Preparation:

Weigh accurately 124 mg of Clindamycin phosphate WS and 200mg of Miconazole nitrate WS in a 100 ml volumetric flask, add 70 ml of diluent, shake well to dissolve and make up the volume to 100ml with diluent. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Sample Preparation:

Collect the Medicament, take one capsule fill weight in a 100 ml volumetric flask, and add 30 ml of diluent, shake well to dissolve and make up the volume to 100 ml with diluent. Mix well and Filter. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Injection sequence:

Inject the solutions as per the following sequence

Solution Name	No of injections	Purpose
Diluent	1	Blank
Standard solution	6	System suitability &Quantification
Sample solution-1	1	To verify the robustness
Sample solution-2	1	To verify the robustness

Acceptance criteria:

System Suitability Parameters	**Acceptance criteria**
Tailing factor for Clindamycin Phosphate and Miconazole peak from first standard injection.	**NMT 2.0**

Theoretical plate count for Clindamycin Phosphate and Miconazole peak from first standard injection.	**NLT 2000**
The %RSD of RT for six injection of standard solution	**NMT 1.0**
The %RSD of Peak response for six injection of standard solution	**NMT 2.0**
% of Assay 1) At 208 nm 2) At 210 nm 3) At 212 nm	**NLT 90.0**
The % difference between the Assay results obtained with three different wavelengths.	**NMT 2.0**

FILTER VALIDATION:

Filter validation of the analytical method will be demonstrated by assaying the homogeneous sample in duplicate (without filtration and with filtration) of the sample solution. The percentage RSD of the test results will be calculated.

Filter validation will be demonstrated by assaying the sample with out filtration by centrifuging the sample solution, filtering through 0.45µm nylon filter (millex) and filtering the solution by Whatman filter paper no.42.

Standard Preparation:

Weigh accurately 124 mg of Clindamycin phosphate WS and 200mg of Miconazole nitrate WS in a 100 ml volumetric flask, add 70 ml of diluent, shake well to dissolve and make up the volume to 100ml with diluent. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Sample Preparation:

Collect the Medicament, take one capsule fill weight in a 100 ml volumetric flask, and add 30 ml of diluent, shake well to dissolve and make up the volume to 100 ml with diluent. Mix well and Filter. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Solution Without filtration:

Centrifuge the sample solution and inject.

Whatman filter paper no: 42:

Filter the sample solution with Whatman filter paper no: 42 and inject.

0.45µm nylon filter.

Filter the sample solution with 0.45µm nylon filter and inject

Injection sequence

Inject the solutions as per the following sequence:

Solution Name	No of injections	Purpose
Diluent	1	Blank
Standard solution	6	System suitability &Quantification
Sample solution without filtration(Centrifuged	1	For filtration study

solution) 2 Preparation each		
Sample solution filtered with Whatman filter paper no: 42 2 Preparation each	1	
Sample solution filtered with 0.45µm nylon filter 2 Preparation each	1	

Acceptance criteria:

System Suitability Parameters	**Acceptance criteria**
Tailing factor for Clindamycin and Miconazole peak from first standard injection.	**NMT 2.0**
Theoretical plate count for Clindamycin and Miconazole peak from first standard injection.	**NLT 2000**
The %RSD of Peak response for six injection of standard solution solution	**NMT 2.0**
% of Assay 1. Sample solution without filtration(Centrifuged) 2. Sample solution filtered with Whatman filter paper no: 42	**NLT 90.0**

3.Sample solution filtered with 0.45µm nylon filter	
The % difference between the Assay results obtained with three different filters	**NMT 2.0**

STABILITY OF ANALYTICAL SOLUTIONS:

Stability of standard & sample solution will be demonstrated by injecting standard & sample solution with different time interval from the time of preparation. Solutions shall be injected once in 4 hours till the completion of 48 hours. The stability of solution shall be decided based on the area obtained at different time interval. This test shall be stopped at any time interval, if the obtained value is not meeting the acceptance criteria.

Standard Preparation:

Weigh accurately 124 mg of Clindamycin phosphate WS and 200mg of Miconazole nitrate WS in a 100 ml volumetric flask, add 70 ml of diluent, shake well to dissolve and make up the volume to 100ml with diluent. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Sample Preparation:

Collect the Medicament, take one capsule fill weight in a 100 ml volumetric flask, and add 30 ml of diluent, shake well to dissolve and make up the volume to 100 ml with diluent. Mix well and Filter. Pipette out 10ml solution to a 100.0 ml volumetric flask, make up volume with diluent.

Injection sequence:

Inject the solutions as per the following sequence

Solution Name	No of injections	Purpose	Time
Diluent	1	Blank	Sequence shall be started at Every 4 hours
Standard solution	1	To verify the stability	
Sample solution-1	1		

Acceptance criteria:

System Suitability Parameters	**Acceptance criteria**
Tailing factor for Clindamycin and Miconazole peak in standard injection.	**NMT 2.0**
Theoretical plate count for Clindamycin and Miconazole peak in standard injection.	**NLT 2000**
For a stable solution the RSD of the peak area	**NMT 2.0 %**

6.RESULTS AND DISCUSSIONS :

SYSTEM SUITABILITY:

Injecting Clindamycin Standard and Miconazole nitrate Solution performs system suitability six times. The calculated Summarized results listed below

For Clindamycin:

No of Injection	**RT**	**Response**	**Tailing factor**	**Theoretical plate**
01	2.40	174539	1.1	4248
02	2.40	172974	1.1	4210
03	2.40	172944	1.1	4143
04	2.40	172789	1.1	4148
05	2.39	173749	1.1	4295
06	2.40	172570	1.1	4341
Mean	2.40	173261	1.13	4231
SD	0.00	742	0.0	79.4
%RSD	0.17	0.4	0.7	1.9

For Miconazole nitrate :

No of Injection	RT	Response	Tailing factor	Theoretical plate
01	8.92	6670321	1.5	5409
02	8.9	6655200	1.5	5389
03	8.89	6653601	1.4	5379
04	8.89	6647653	1.5	5451
05	8.89	6647350	1.4	5385
06	8.89	6642687	1.4	5465
Mean	8.90	6652802	1.44	5413.0
SD	0.0	9714.5	0.0	36.6
%RSD	0.1	0.1	1.6	0.7

SPECIFICITY:

The Specificity established by injecting the following solutions

- Blank
- Placebo solution
- Standard solution
- Sample solution

The calculated Summarized results listed below.

	RT	**Area**	**Peak purity index**
Blank	NA	NA	NA
Placebo	NA	NA	NA
Working standard Clindamycin	2.456	178493	0.99
Miconazole Nitrate	9.065	6664107	0.99
Sample Clindamycin	2.413	182272	0.99
Miconazole nitrate	8.9	6875795	0.99

Acceptance Criteria:

1. There should not be any interference by blank, Placebo peaks with the main analyte peak.
2. The Peak purity index of standard peak in sample should not be less than 0.9990 (For LC Solution software).

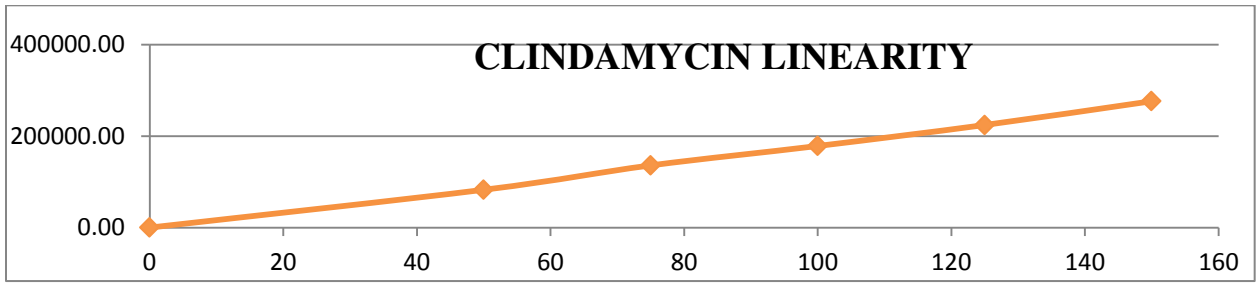
LINEARITY AND RANGE:

The linearity of the method is establishing by performing 5 test concentrations from 50.0% to 150% of working concentrations. Assessed Linearity 50%, 75%, 100%, 125% and 150% each concentration 3 injection performed. The calculated Summarized results listed below.

Linearity for Clindamycin:

Sample	Weight in mg	Volume in mL	Volume in mL	Volume in mL	Peak Area	Conc. in %
Sample 1	124.61	100	5mL →	100mL	82660	50
Sample 2			7.5mL →		136013	75
Sample 3			10mL→		178432	100
Sample 4			12.5mL →		224327	125
Sample 5			15mL →		276335	150

Chart 1 – Linearity for Clindamycin: 50.0 – 150.0% w/v concentration R / peak area units with upper and lower 95.0% confidence limits.



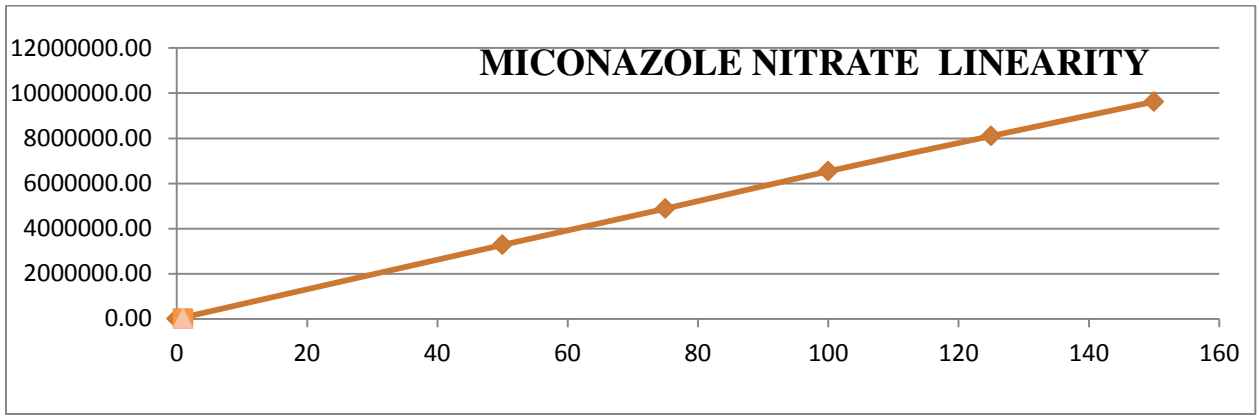
Acceptance criteria:

System Suitability Parameters	Observed value	Acceptance criteria
Correlation coeff. r^2	**0.999**	**NLT 0.999**

Linearity for Miconazole nitrate:

Sample	Weight in mg	Volume in mL	Volume in mL	Volume in mL	Peak Area	Conc. in %
Sample 1	200.77	100	5mL →	100mL	3276552	50
Sample 2			7.5mL →		4885767	75
Sample 3			10mL→		6539879	100
Sample 4			12.5mL →		8102161	125
Sample 5			15mL →		9622955	150

Chart 1 – Linearity for Miconazole nitrate**:** 50.0 – 150.0% w/v Concentration R / peak area units with upper and lower 95.0% confidence limits.



Acceptance criteria:

System Suitability Parameters	Observed value	Acceptance criteria
Correlation coeff. r^2	**0.999**	**NLT 0.999**

ACCURACY:

Accuracy is assessed using a '9' determination i.e., '3' concentration (50%, 100%, and 150% each Concentration 3 determination).

The calculated Summarized results listed below

FOR CLINDAMYCIN:

Sample #	Addition of Active to Excipients mixture	Placebo Added in mg	Standard added in mg	Recovery in %	Average in %	RSD in %
01	50.0%	1469.72	62.22	101.3	**101.1**	**1.2**
	50.0%	1460.51	62.95	100.2		
	50.0%	1473.13	62.7	101.9		
02	100.0%	1450.07	124.71	99.6	**99.9**	**1.0**
	100.0%	1444.14	124.36	100.6		
	100.0%	1446.56	124.44	99.1		
03	150.0%	1447.40	186.61	98.9	**100.0**	**0.9**
	150.0%	1445.05	186.22	99.4		
	150.0%	1451.8	186.79	100.7		
				Average	**100.3**	**1.0**
				Limit	**98.0– 102.0**	**RSD NMT2.0**
				Conclusion	**Complies**	**Complies**

FOR MICONAZOLE NITRATE:

Sample #	Addition of Active to Excipients mixture	Placebo Added in mg	Standard added in mg	Recovery in %	Average in %	RSD in %
01	50.0%	1469.72	100.17	101.5	**100.6**	**0.2**
	50.0%	1460.51	100.57	100.4		
	50.0%	1473.13	100.13	100.7		
02	100.0%	1450.07	200.7	98.6	**101.5**	**0.2**
	100.0%	1444.14	200.07	101.6		
	100.0%	1446.56	200.45	101.3		
03	150.0%	1447.40	300.13	98.6	**100.3**	**0.6**
	150.0%	1445.05	299.7	99.9		
	150.0%	1451.8	300.46	100.7		
				Average	**100.8**	**0.3**
				Limit	**98.0– 102.0**	**RSD NMT2.0**
				Conclusion	**Complies**	**Complies**

Acceptance criteria:

System Suitability Parameters	Observed value	Acceptance criteria
Tailing factor for Clindamycin, Miconazole nitrate peak from first standard injection.	1.1 1.5	**NMT 2.0**
Theoretical plate count for Clindamycin, Miconazole nitrate peak from first standard injection.	3850 4853	**NLT 2000**
The %RSD of RT for six injection of standard solution 1)Clindamycin 2) Miconazole nitrate	 0.0 0.1	 **NMT 1.0**
The %RSD of Peak response for six injection of standard solution 1)Clindamycin 2) Miconazole nitrate	 1.3 0.1	 **NMT 2.0**
The Average % of recovery 1)Clindamycin 2) Miconazole nitrate	 100.3 100.8	 **98.0 to 102.0**

The Average % RSD of Recovery 1)Clindamycin 2) Miconazole nitrate	 1.0 0.3	 **NMT 2.0**

PRECISION:

System Precision:

System precision will be tested by injecting 6 replicate injections of Clindamycin, and Miconazole nitrate working standard solution. The calculated Summarized results listed below.

For Clindamycin:

No. of Injection	**RT**	**Response**	**Tailing factor**	**Theoretical plate**
01	2.40	174539	1.1	4248
02	2.40	172974	1.1	4210
03	2.40	172944	1.1	4143
04	2.40	172789	1.1	4148
05	2.39	173749	1.1	4295
06	2.40	172570	1.1	4341
Mean	**2.40**	**173261**	**1.13**	**4231**

SD	**0.00**	**742**	**0.0**	**79.4**
%RSD	**0.17**	**0.4**	**0.7**	**1.9**

For Miconazole nitrate

No of Injection	**RT**	**Response**	**Tailing factor**	**Theoretical plate**
01	8.92	6670321	1.5	5409
02	8.9	6655200	1.5	5389
03	8.89	6653601	1.4	5379
04	8.89	6647653	1.5	5451
05	8.89	6647350	1.4	5385
06	8.89	6642687	1.4	5465
Mean	**8.90**	**6652802**	**1.44**	**5413.0**
SD	**0.0**	**9714.5**	**0.0**	**36.6**
%RSD	**0.1**	**0.1**	**1.6**	**0.7**

Acceptance criteria:

System Suitability Parameters	**Observed value**		**Acceptance criteria**
	Clindamycin	**Miconazole nitrate**	
Tailing factor from first	1.1	1.5	**NMT 2.0**

standard injection.			
Theoretical plate count from first standard injection.	4248	5409	**NLT 2000**
The % RSD of RT for six injection of standard solution	0.0	0.1	**NMT 1.0**
The % RSD of Peak response for six injection of standard solution	0.4	0.1	**NMT 2.0**

Method Precision:

Repeatability of the method performed by injecting 6 replicate injections of Clindamycin 100mg, Miconazole nitrate 200mg capsules Sample solution. The calculated Summarized results listed below.

Acceptance criteria:

System Suitability Parameters	**Observed value**		**Acceptance criteria**
	Clindamycin	**Miconazole nitrate**	
Tailing factor from first standard injection.	1.2	1.7	**NMT 2.0**
Theoretical plate count from	4371	8665	**NLT 2000**

first standard injection.			
The % RSD of RT for six injection of standard solution	0.5	0.4	**NMT 1.0**
The % RSD of Peak response for six injection of standard solution	0.8	0.7	**NMT 2.0**
% of Assay	123.1	104.7	**NLT 90.0**
% RSD of Assay of 6 replicate sample	1.5	1.0	**NMT 2.0**

Intermediate Precision:

Intermediate precision will be demonstrated by analyzing the same Clindamycin 100mg, Miconazole nitrate 200mg Capsules batch as above in 6 replicate samples, in the same lab but by a different Analyst, using a different Instrument and on a different day.

Acceptance criteria:

System Suitability Parameters	**Observed value**		**Acceptance criteria**
	Clindamycin	**Miconazole nitrate**	
Tailing factor from first standard injection. 1.Analyst-1	1.1 1.2	1.5 1.7	**NMT 2.0**

2.Analyst-2			
Theoretical plate count from first standard injection. 1.Analyst-1 2.Analyst-2	 4248 4371	 5409 8665	**NLT 2000**
The % RSD of RT for six injection of standard solution 1.Analyst-1 2.Analyst-2	 0.0 0.5	 0.1 0.4	**NMT 1.0**
The % RSD of Peak response for six injection of standard solution 1.Analyst-1 2.Analyst-2	 0.4 0.8	 0.1 0.7	**NMT 2.0**
% of Assay 1.Analyst-1 2.Analyst-2	123.9 123.1	103.3 104.7	**NLT 90.0**

Confidence limits 1.Analyst-1 2.Analyst-2	1.0 1.5	1.1 0.9	**±5%**
% RSD of Assay of 6 replicate samples by Analyst-1 Analyst-2	1.0 1.5	1.4 1.0	**NMT 2.0**
% RSD of Assay of 12 replicate samples by Analyst-1 & Analyst-2	1.3	1.3	**NMT 2.0**

RUGGEDNESS:

The Ruggedness has been demonstrated by injecting six samples prepared as per the test preparation given the method of analysis on two different days with different analysts and different system (minimum two different system).Calculate the mean, standard deviation, relative standard deviation and confidence limits from six test preparations.

System to System variability:

The Ruggedness has been demonstrated by injecting six samples prepared as per the test preparation given the method of analysis on two different days with different analysts and different system (minimum two different system).Calculate the mean, standard deviation, relative standard deviation and confidence limits for Assay from six test preparations. Summarize the results in the table given below.

Acceptance criteria:

System Suitability Parameters	Observed value		Acceptance criteria
	Clindamycin	**Miconazole nitrate**	
Tailing factor from first standard injection. 1) System-1 2) System-2	 1.1 1.2	 1.5 1.7	**NMT 2.0**
Theoretical plate count from first standard injection. 1) System-1 2) System-2	 4248 4371	 5409 8665	**NLT 2000**
The % RSD of RT for six injection of standard solution 1) System-1 2) System-2	 0.0 0.5	 0.1 0.4	**NMT 1.0**
The % RSD of Peak response for six injection of standard solution 1) System-1	 0.4	 0.1	

2) System-2	0.8	0.7	**NMT 2.0**
% of Assay 1) System-1 2) System-2	123.9 123.1	103.3 104.7	**NLT 90.0**
Confidence limits 1) System-1 2) System-2	1.0 1.5	1.1 0.9	**±5%**
% RSD of Assay of 6replicate samples by 1) System-1 2) System-2	1.0 1.5	1.4 1.0	**NMT 2.0**
% RSD of Assay of 12 replicate samples by System-1 & System-2	1.3	1.3	

Column to Column variability:

The Ruggedness has been demonstrated injecting six samples prepared as per the test preparation given the method of analysis on two different days with different analysts and different column (minimum two different columns). Calculate the mean, standard deviation, relative standard deviation and confidence limits for Assay from six test preparations. Summarize the results in the table given below.

System Suitability Parameters	Observed value		Acceptance criteria
	Clindamycin	Miconazole nitrate	
Tailing factor from first standard injection. 1) Column-1 2) Column-2	1.1 1.2	1.5 1.7	**NMT 2.0**
Theoretical plate count from first standard injection. 1) Column-1 2) Column-2	4248 4371	5409 8665	**NLT 2000**
The % RSD of RT for six injection of standard solution 1) Column-1 2) Column-2	0.0 0.5	0.1 0.4	**NMT 1.0**
The % RSD of Peak response for six injection of standard solution		0.1	

1) Column-1 2) Column-2	0.4 0.8	0.7	**NMT 2.0**
% of Assay 1) Column-1 2) Column-2	123.9 123.1	103.3 104.7	**NLT 90.0**
Confidence limits 1) Column-1 2) Column-2	1.0 1.5	1.1 0.9	**±5%**
% RSD of Assay of 6replicate samples by 1) Column-1 2) Column-2	1.0 1.5	1.4 1.0	**NMT 2.0**
% RSD of Assay of 12 replicate samples by Column-1 & Column-2	1.3	1.3	

ROBUSTNESS:

The Robustness will be demonstrated for Clindamycin, and Miconazole nitrate Assay analytical method by changing chromatographic parameters. Parameters that influence the variations are flow rate and wavelengths.

Effect of variation in Mobile phase composition

To demonstrate the robustness, check the system suitability parameters by injecting standard preparation, by using two mobile phases, one containing 95% of the method organic phase composition and other containing 105% of the method organic phase composition.

Acceptance criteria:

System Suitability Parameters	**Observed value**		**Acceptance criteria**
	Clindamycin	**Clindamycin**	
Tailing factor for Clindamycin Phosphate and Miconazole peak from first standard injection. 1) 95% methanol composition 2) 100% methanol composition 3) 105% methanol composition	1.4 1.2 1.3	1.5 1.7 1.5	**NMT 2.0**
Theoretical plate count for Clindamycin Phosphate and Miconazole peak from first standard injection. 1) 95% methanol composition 2) 100% methanol composition	3295 4371	8126 8665	**NLT 2000**

3)105% methanol composition	3258	8055	
The %RSD of RT for six injection of standard solution 1) 95% methanol composition 2) 100% methanol composition 3)105% methanol composition	 0.0 0.5 0.3	 0.1 0.1 0.2	**NMT 1.0**
The %RSD of Peak response for six injection of standard solution 1) 95% methanol composition 2) 100% methanol composition 3)105% methanol composition	 0.4 0.8 0.7	 0.1 0.1 0.1	**NMT 2.0**
% of Assay 1) 95% methanol composition 2) 100% methanol composition 3) methanol composition	 124.5 124.4 124.0	 103.7 105.8 104.3	**NLT 90.0**

The % difference between the Assay results obtained with three different mobile phase composition	0.2	1.0	**NMT 2.0**

Effect of variation in flow rate:

To demonstrate the robustness of test method, check the system suitability parameters by injecting standard preparations into the HPLC system with 1.3 ml/min, 1.5 ml/min and 1.7 ml/min.

System Suitability Parameters	**Observed value**		**Acceptance criteria**
	Clindamycin	**Miconazole nitrate**	
Tailing factor from first standard injection. 1) 1.3ml/min 2) 1.5ml/min 3) 1.7ml/min	 1.2 1.1 1.2	 1.7 1.5 1.7	**NMT 2.0**
Theoretical plate count from first standard injection. 1) 1.3ml/min 2) 1.5ml/min	 4646 4248	 9132 5409	**NLT 2000**

3) 1.7ml/min	3982	8209	
The % RSD of RT for six injection of standard solution 1) 1.3ml/min 2) 1.5ml/min 3) 1.7ml/min	 0.1 0.0 0.2	 0.1 0.1 0.1	 **NMT 1.0**
The % RSD of Peak response for six injection of standard solution 1) 1.3ml/min 2) 1.5ml/min 3) 1.7ml/min	 0.4 0.8 0.2	 0.1 0.7 0.1	 **NMT 2.0**
% of Assay 1) 1.3ml/min 2) 1.5ml/min 3) 1.7ml/min	 124.9 123.9 125.2	 104.7 103.3 104.4	 **NLT 90.0**
The % RSD difference between the Assay results obtained with	0.3	0.7	**NMT 2.0**

three different flow rates			

Effect of variation in Wavelength:

To demonstrate the robustness of **t**est method, check the system suitability parameters by injecting standard preparations into the HPLC system with 208nm 210nm and 212nm Wavelength. Calculate the mean, standard deviation and relative standard deviation for Assay from six test preparations. Summarize the results in the table given below.

System Suitability Parameters	**Observed value**		**Acceptance criteria**
	Clindamycin	**Miconazole nitrate**	
Tailing factor from first standard injection. 1) 208nm 2) 210nm 3) 212nm	 1.1 1.1 1.1	 1.3 1.5 1.2	**NMT 2.0**
Theoretical plate count from first standard injection. 1) 208nm 2) 210nm 3) 212nm	 4193 4248 4113	 5341 5409 5477	**NLT 2000**
The % RSD of RT for six injection of standard			

solution 1) 208nm 2) 210nm 3) 212nm	 0.0 0.0 0.0	 0.1 0.1 0.1	 **NMT 1.0**
The % RSD of Peak response for six injection of standard solution 1) 208nm 2) 210nm 3) 212nm	 0.7 0.8 1.8	 0.1 0.7 0.2	 **NMT 2.0**
% of Assay 1) 208nm 2) 210nm 3) 212nm	 123.6 123.9 123.4	 103.0 103.3 103.2	 **NLT 90.0**
The % RSD difference between the Assay results obtained with three different wavelengths	0.5	0.1	**NMT 2.0**

FILTER VALIDATIONS:

Filter validation will be demonstrated by Assay the sample without filtration by centrifuging the sample solution, filtering through 0.45µm nylon filter (millex) and filtering the solution by Whatman filter paper no.42.The percentage RSD of the test results will be calculated.

System Suitability Parameters	**Observed value**		**Acceptance criteria**
	Clindamycin	**Miconazole nitrate**	
Tailing factor from first standard injection.	1.1	1.5	**NMT 2.0**
Theoretical plate count from first standard injection.	4248	5409	**NLT 2000**
The % RSD of RT for six injection of standard solution	0.2	0.1	**NMT 1.0**
The % RSD of Peak response for six injection of standard solution	0.4	0.1	**NMT 2.0**
% of Assay 1) Sample solution without filtration (Centrifuged) 2) Sample solution filtered	124.5	103.8	**NLT 90.0**

with Whatman filter paper no: 42 3) Sample solution filtered with 0.45µm nylon filter	126.2 126.2	104.4 104.7	
The % difference between the Assay results obtained with three different filters.	0.8	0.4	**NMT 5.0**

STABILITY OF ANALYTICAL SOLUTIONS:

The solution stability performed by injecting a homogeneous sample solution for every four hours interval up to 48 hours. The % RSD of all test areas was calculated.

Time Intervals	**Clindamycin**		**Miconazole nitrate**	
	Standard	**Sample**	**Standard**	**Sample**
Intial	174539	219967	6670321	6887856
4 Hour	173692	220709	6693139	6923832
8 Hour	174461	218134	6737984	6882313
12 Hour	173764	214756	6722905	6831903
16 Hour	178620	219518	6769786	6829966
20 Hour	178897	212560	6786985	6858255

24 Hour	173115	211639	6798956	6919299
28 Hour	170797	213494	6847856	6959961
32 Hour	173122	215321	6898526	7012512
36 Hour	171526	214352	6948956	7052636
40 Hour	168223	211256	6985265	7089653
44 Hour	167996	207256	7078125	7195623
48 hour	170321	209896	7025121	7187854
Average	173006	214528	6805516	6931653
Std dev	3342	4131	127858	121900
RSD (%)	1.9	1.9	1.9	1.8

Acceptance Criteria:

System Suitability Parameters	**Observed value**		**Acceptance criteria**
	Clindamycin	**Miconazole nitrate**	
Tailing factor from first standard injection.	1.1	1.5	**NMT 2.0**
Theoretical plate count from first standard injection.	4248	5409	**NLT 2000**

For a stable solution the RSD of the peak area not more than 2.0%.(Up to 48 hour) 1) Standard 2) Sample	 1.9 1.9	 1.9 1.8	 **NMT 2.0**

DISCUSSION :

From the above data it was found that the assay value of the Clindamycin and miconazole nirtate sample was found to be 102.3% and 101.3 %. And the method was validated , validation data as follows,

The system suitability parameters reveal that the values were within the specified limits for the proposed method. From the results shown in precision , it was found that % RSD is less than 2%; which indicates that the proposed method has good reproducibility. specificity study reveals that the buffers and degradation products present in the pharmaceutical formulations were not interfering the proposed method. From the linearity Table, it was found that the drug obeys linearity with in the concentration range for Clindamycin and miconazole nirtate , From the results shown in accuracy Table , it was found that the percentage recovery values of pure drug were in between 99.0 to 100.1, indicates that the method was accurate .

From the results shown in the ruggedness data it was observed that bench top stability of standard and test sample was not stable for even one day, so should use immediately. And the refrigerator stability of standard and test sample was not stable for even one day, so should use immediately. bench top stability of mobile phase was with in the limits for up to two days.from the robustness data we found that mobile phase variation (ethanol or acetonitrile) was accepted from 90-110%, variation in the ph was accepted from 5.5 to 5.9, column oven temperature was between 35^0 to 45^0 , filter also validatd , found that there is no filter interference from the filtered and centrifuged samples. So filters are suitable for filtration

The Test procedure for Clindamycin and miconazole nirtate capsule was validated and found to be linear, Specific, Precise, Accurate, Rugged and robust.

8. SUMMARY:

Parameter	Experiment	Observation	Acceptance criteria
System suitability	% RSD Clindamycin Miconazole nitrate	 0.4 0.1	The % RSD should be NMT 2.0
Specificity	Placebo interference Placebo Interference and Degradation products	Complies Complies	The Placebo should not show any peak at the retention time of Clindamycin, Miconazole nitrate, peak. The Purity angle index should be 0.999(For LC Solution Software).
Linearity and Range	Coefficient of correlation (r^2)	0.999	The correlation co-efficient (r) should not less than 0.999.
Accuracy	Recovery Clindamycin Miconazole nitrate	 100.3 and 1.0 100.8 and 0.3	The % Recovery should not be less than 98.0% and not more than 102.0%.The % RSD should not be more than 2.0.
Precision	System Precision Clindamycin Miconazole nitrate	 0.4 0.1	The % RSD should be NMT 2.0

	Method Precision Clindamycin Miconazole nitrate	 1.0 and 1.0 1.4 and 1.1	The % RSD of Assay value not more than 2.0.The Confidence limits should be ± 5.0%
	Intermediate Precision Clindamycin Miconazole nitrate	 1.5 and 1.5 1.0 and 0.9	The % RSD of Assay value not more than 2.0.The Confidence limits should be ± 5.0%.
Ruggedness	System to System Variability Clindamycin Miconazole nitrate	 1.3 1.3	The % RSD of Assay value
	Column to Column Variability Clindamycin Miconazole nitrate	 1.3 1.3	The % RSD of Assay value 2.0.

Robustness	Effect of variation in Mobile phase composition Clindamycin Miconazole nitrate	0.2 1.0	The % RSD of Assay value
	Effect of variation in flow rate Clindamycin Miconazole nitrate	0.3 0.7	The % RSD of Assay value
	Effect of variation in wavelength Clindamycin Miconazole nitrate	0.5 0.1	The % RSD of Assay value
Filter validation Clindamycin Miconazole nitrate	Centrifugation Whatman filter paper no:42 0.45µm nylon filter	0.8 0.4	The RSD of Test results NMT 2.0.%

Stability of analytical solutions Clindamycin Miconazole nitrate	Standard Sample Solution	1.9 and 1.9 1.9 and 1.8	The RSD of Test results NMT 2.0.%

FIGURES :

SYSTEM SUITABILITY:

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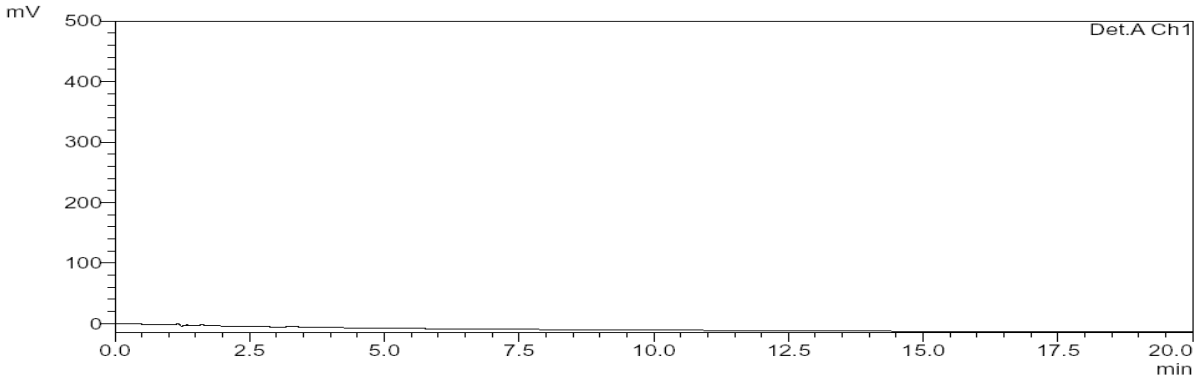

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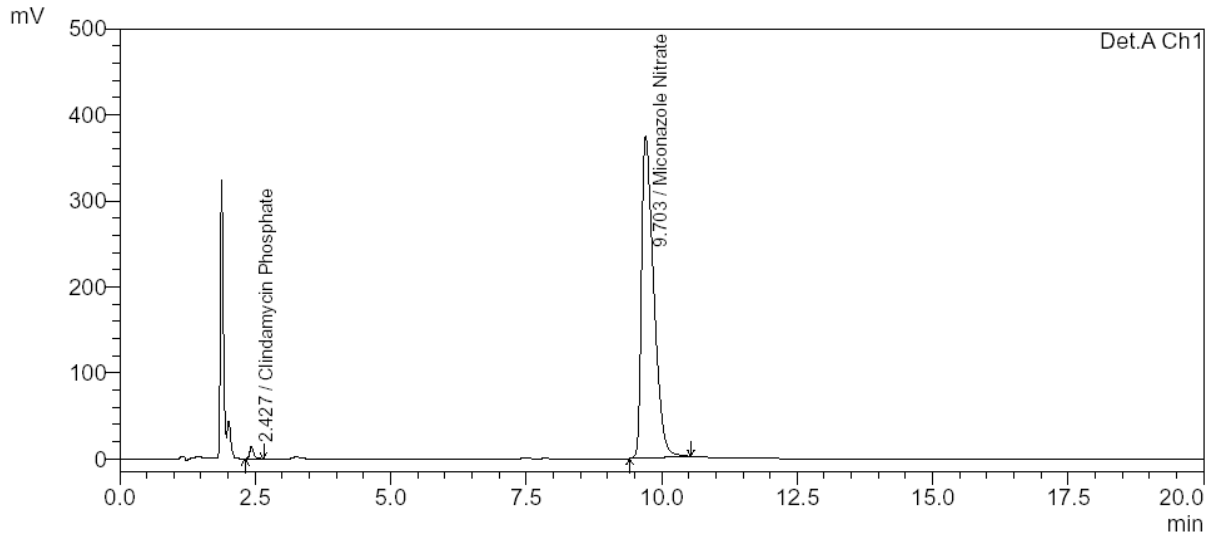

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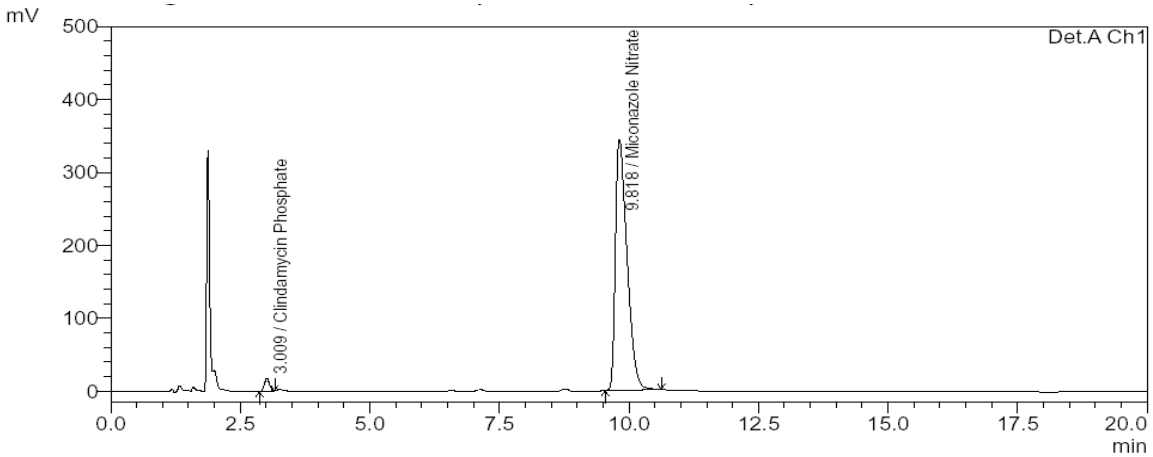

SPECIFICITY:

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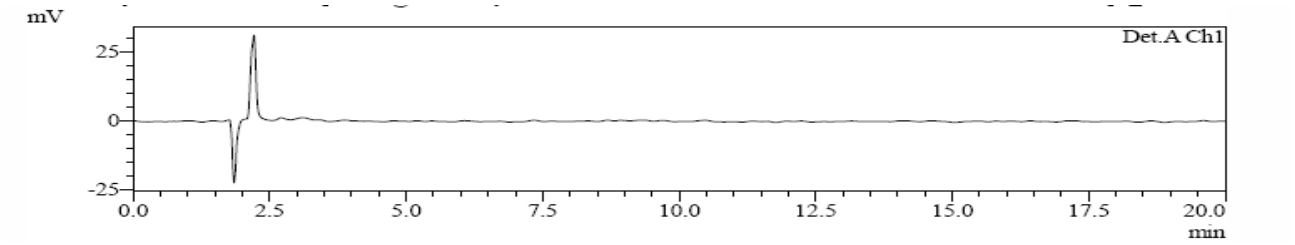

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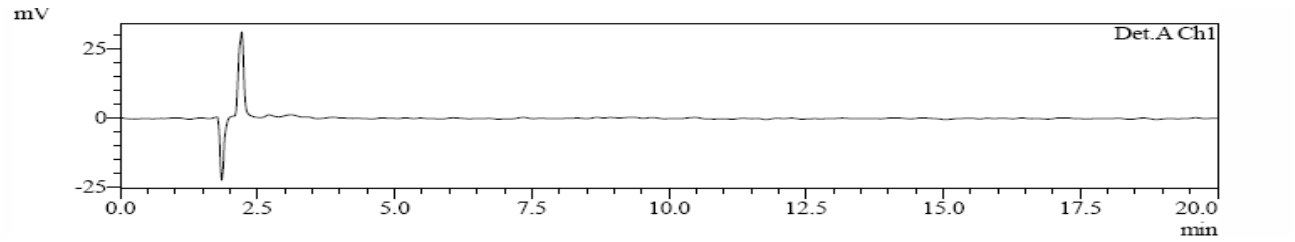

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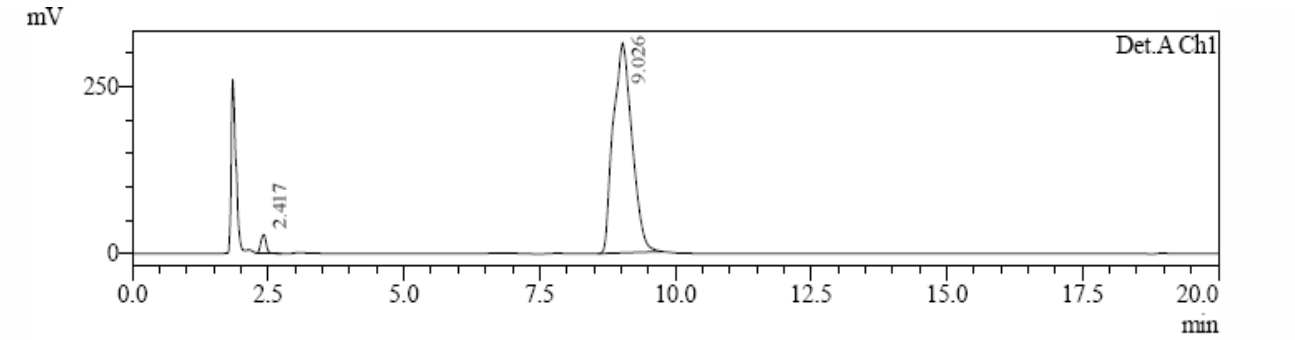
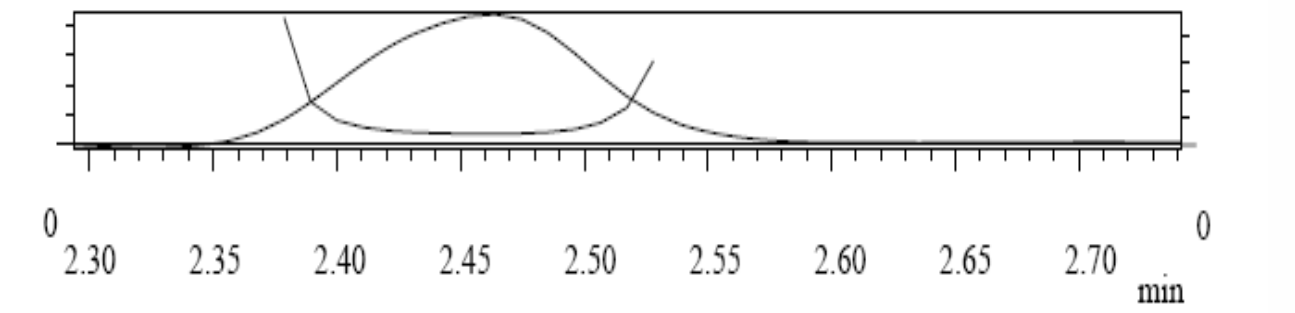
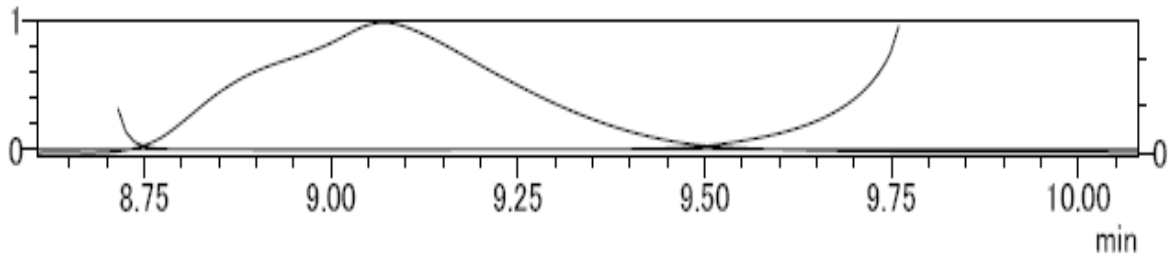
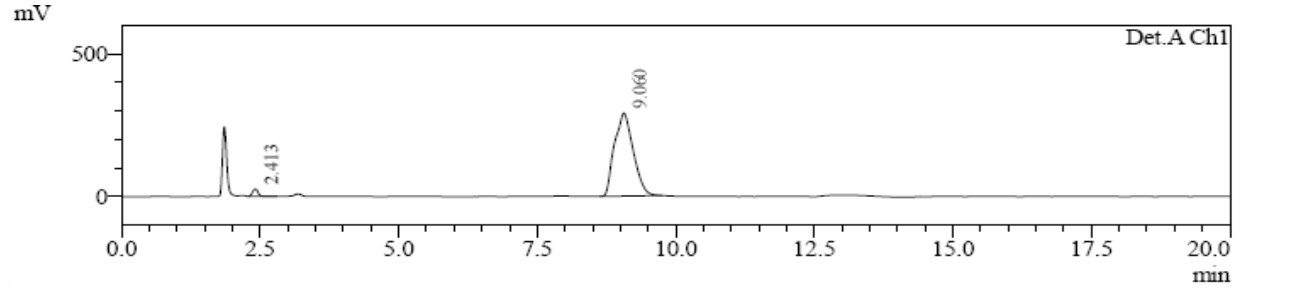
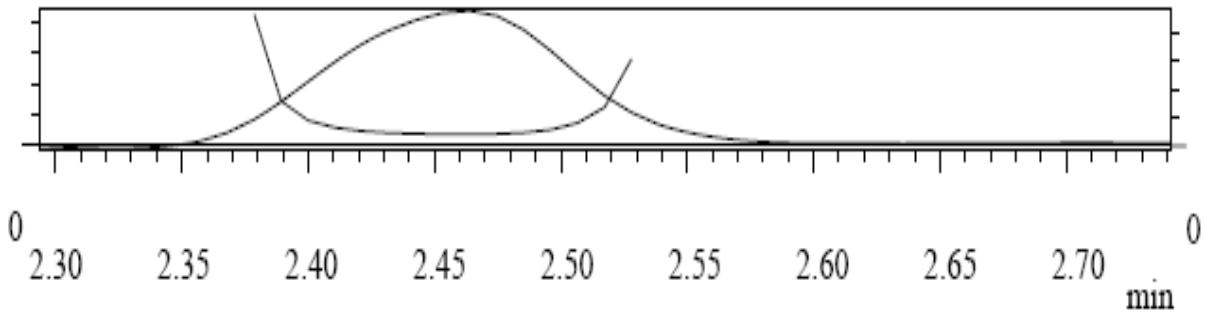





1
0
0
8.75
9.00
9.25
9.50
9.75
10.00
min

Sample:


mV
500
0
Det.A Ch1
2.413
9.060
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
min


0
0
2.30
2.35
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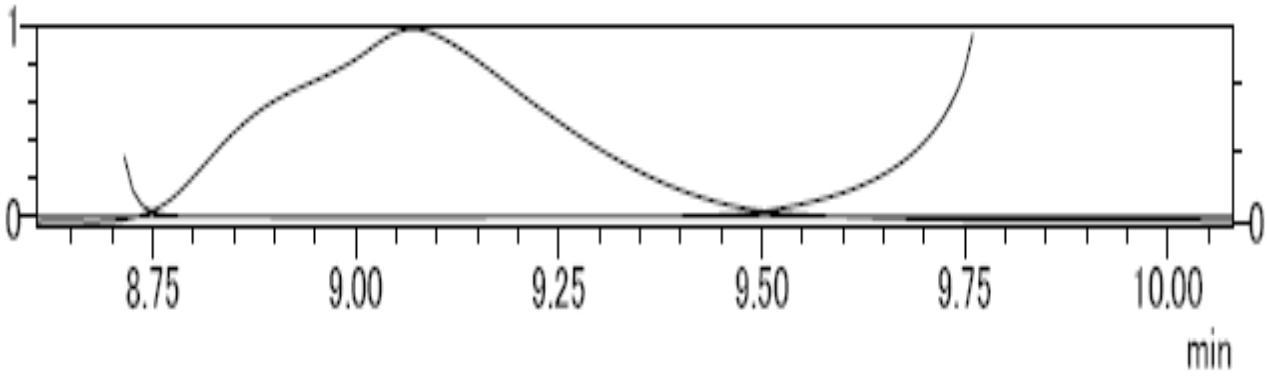
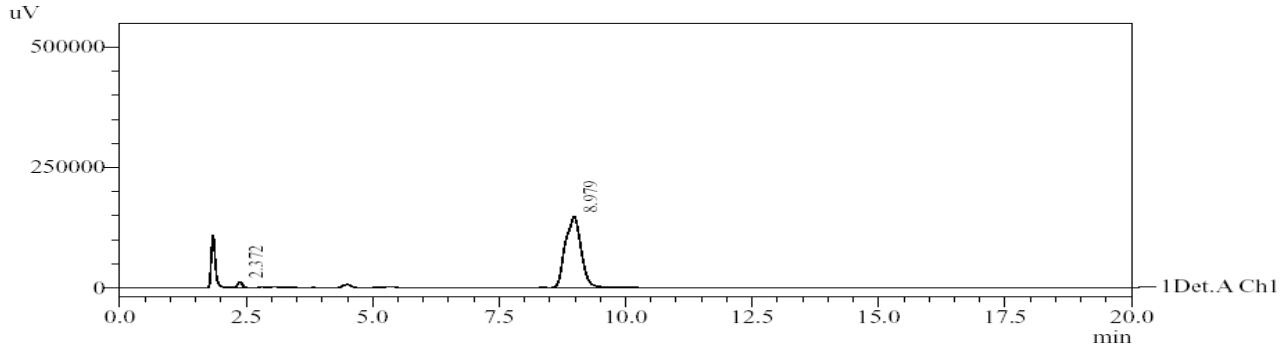
2.45
2.50
2.55
2.60
2.65
2.70
min

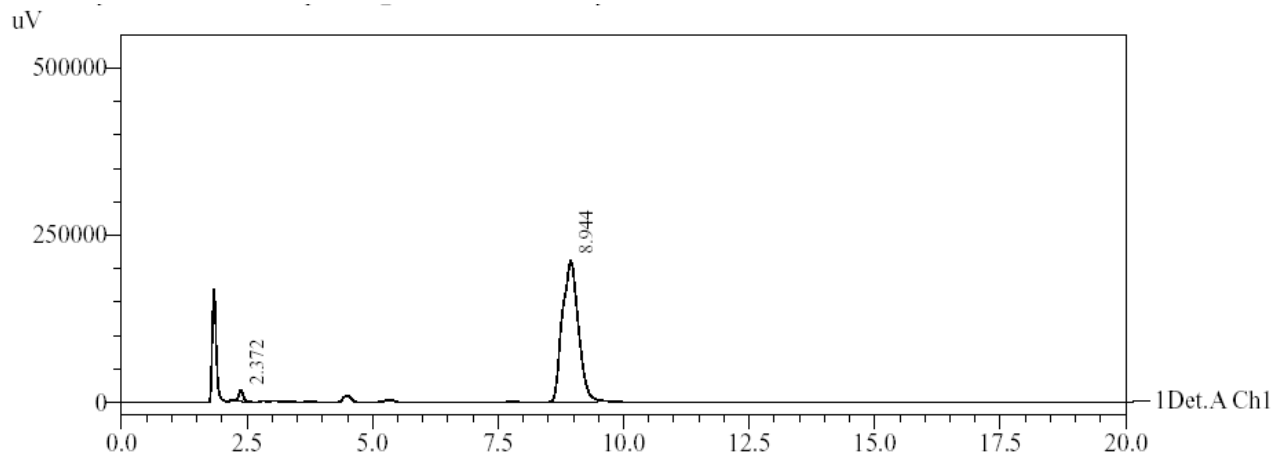

1
0
0
8.75
9.00
9.25
9.50
9.75
10.00
min

LINEARITY

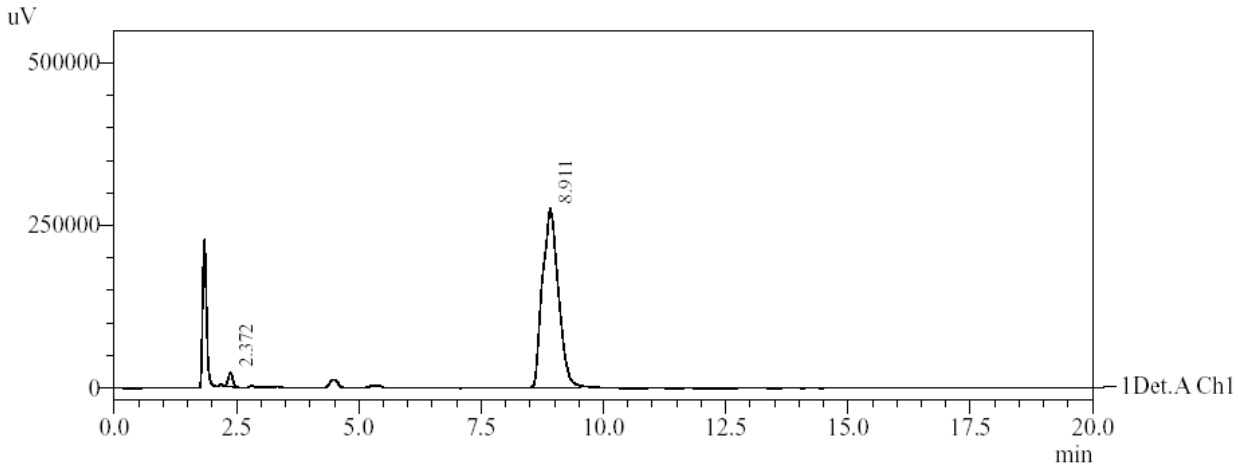
Linearity for 50% Solution:



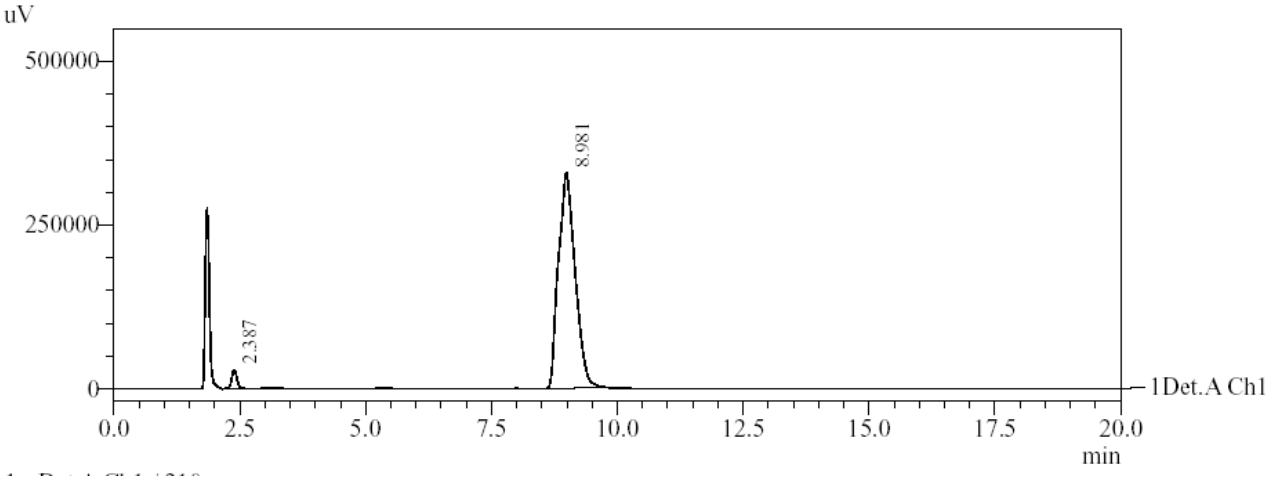
Linearity for 75% Solution:



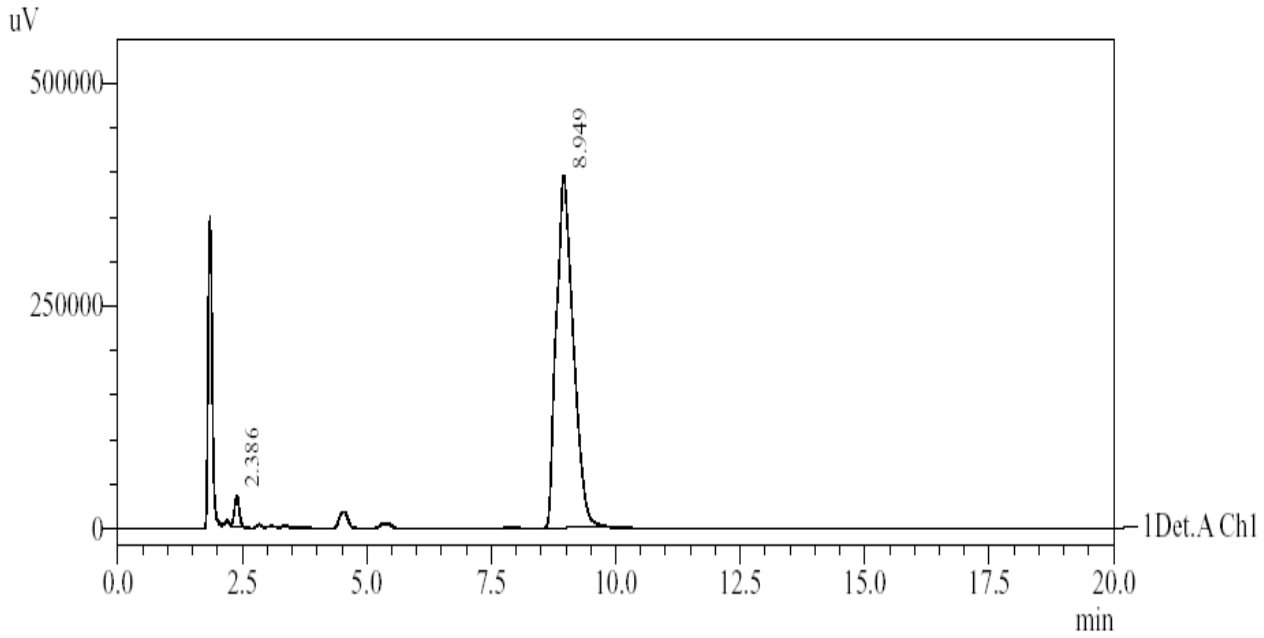
Linearity for 100% Solution:



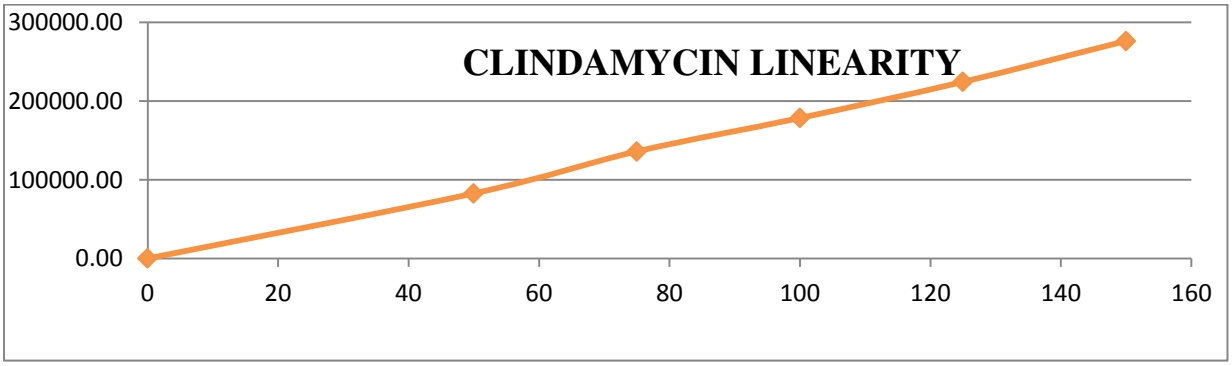
Linearity for 125% Solution:



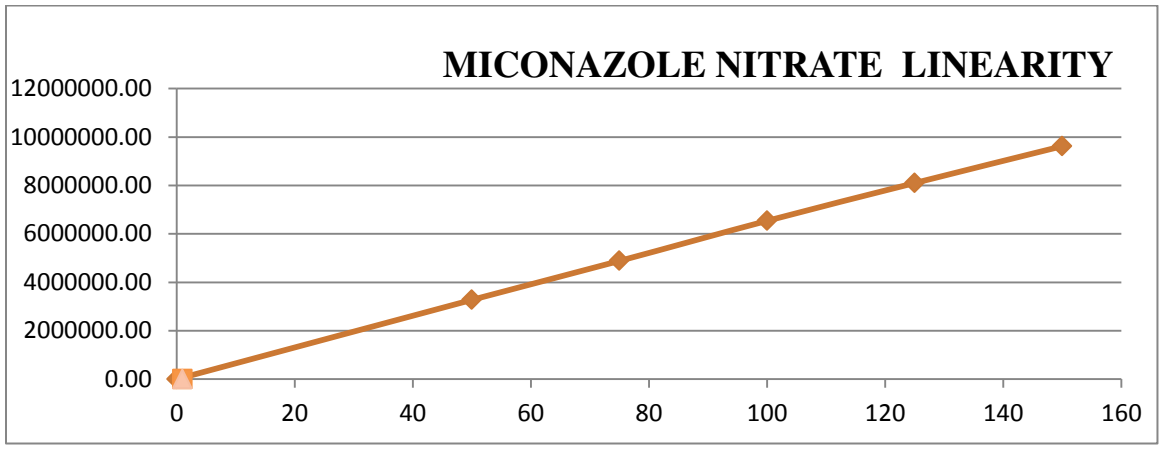
Linearity for 150% Solution:



LINEARITY CURVE FOR CLINDAMYCIN

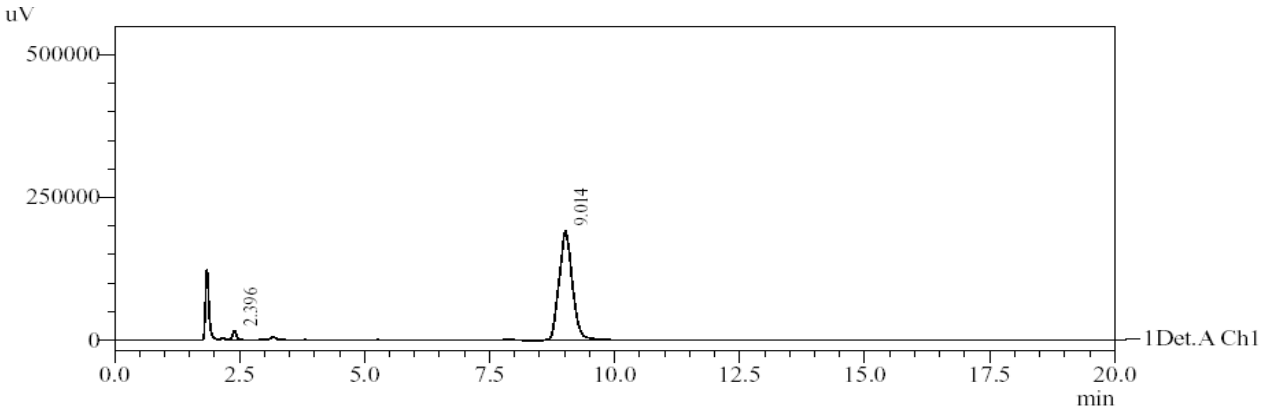


LINEARITY CURVE FOR MICONAZOLE NITRATE:



ACCURACY

Accuracy for 50% Solution:



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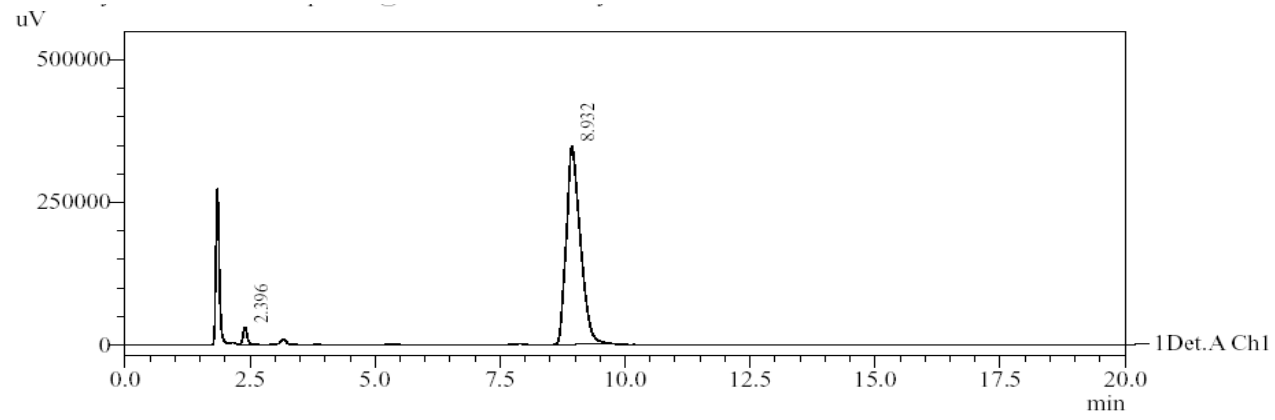
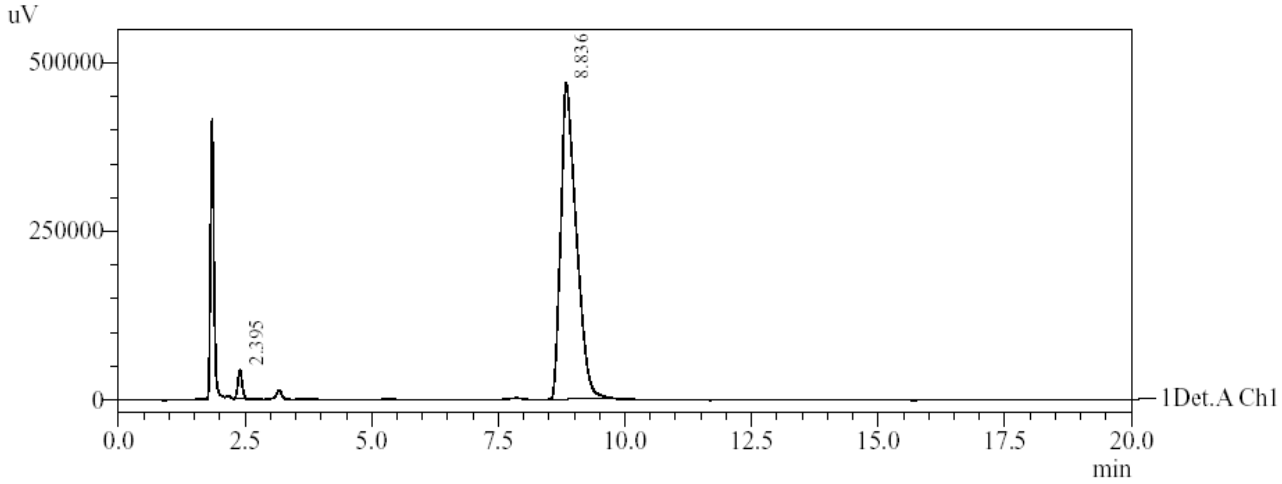
Accuracy 100% Solution:



Accuracy 150% Solution:



9. CONCLUSION :

The proposed analytical method is simple, economical, rapid, reproducible and accurate for the estimation of Clindamycin and Miconazole nitrate Capsules. A newer RP-HPLC method was developed for formulations. The proposed method gives reliable assay results with short analysis time using mobile phase of sodium acetate tri hydrate , acetonitrile &ethanol..

The above method does not suffer from any interference due to common excipients or degradation products. Thus it was show that proposed methods could be successfully applied to estimate commercial pharmaceutical products containing Clindamycin and Miconazole nitrate. Thus the above studies and findings will enable the quantification of the drug for future investigation in the field of analytical chemistry.

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