Chapter One

1. Introduction

Metronidazole

1.1 Metronidazole Overview:

1.1.1History of Metronidazole as an active pharmaceutical ingredient:

Metronidazole was introduced as an antiprotozoal agent but it is also active against anaerobic bacteria such as the species Bacteroides clostridium and Helicobacter species and some Streptococci. It is effective in the therapy of pseudomembranous colitis and a clostridial infection. It is the most effective drug available for invasive amoebiasis involving the intestine or the liver. Metronidazole was found to have particularly high activity invitro and also found to be effective in vivo against Trichomonasvaginalis and Enterobacterhistolytica.

Reported that oral doses of the drug impartedtrichomonacidal activity to semen and urine and that high cure rates could be obtained in bothmale and female patients with trichomoniasis. Recent studies have suggested that metronidazoletherapy may benefit some patients with peptic ulcer who are infected with H. pylori.Because of the increasing complexity of modern pharmaceutical manufacture arising from a variety of unique drugs and dosage forms, complex ethical, legal and economic responsibilities have been placed on those concerned with

manufacture of modern pharmaceuticals. An awareness of these factors is the responsibility of all those involved in the development, manufacture, control and marketing of quality products. The major causes that lead to substandard drugs are given below:

1- Addition of incorrect quantity of active ingredient or date expired sub-potent materials.

2- Non-uniform distribution of active ingredients and Poor stability of active ingredients in the finished product.

3- Substandard or spurious drugs could endanger patient's life.

4- After the implementation of the National Drug Policy in 1982 the quality of marketed drug, no doubt, improved, but not as expected.

5- This realization influenced to evaluate the metronidazole preparations available in the market.

1.1.2 Pharmacokinetic of Metronidazole Tablets:

1.1.2.1Absorption:

Disposition of Metronidazole in the body is similar for both oral and intravenous dosage forms. Following oral administration, Metronidazole is well absorbed, with peak plasma concentrations occurring between one and two hours after administration.

Plasma concentrations ofMetronidazoleareproportional to the administered dose. Oral administration of 250 mg, 500 mg, or 2,000 mg produced peak plasma concentrations of 6 mcg/mL, 12

mcg/mL, and 40 mcg/mL, respectively. Studies reveal no significant bioavailability differences between malesand females; however, becauseofweight differences, theresultingplasmalevels in males aregenerallylower.

1.1.2.2 Distribution:

Metronidazole is the major component appearing in the plasma, with lesser quantities of metabolites also being present. Less than 20% of the circulating Metronidazole is bound to plasma proteins. Metronidazole appears in cerebrospinal fluid, saliva, and breast milk in concentrations similar to those found in plasma. Bactericidal concentrations of Metronidazole have also been detected in pus from hepatic abscesses.

1.1.2.3 Metabolism/Excretion:

The major route of elimination of Metronidazole and its metabolites is via the urine (60% to 80% of the dose), with fecal excretion accounting for 6% to 15% of the dose. The metabolites that appear in the urine result primarily from side-chain oxidation [1-(ßhydroxyethyl)-2-hydroxymethyl-5-nitroimidazoleand 2-methyl-5-nitroimidazole-1-yl acetic acid] and glucuronide conjugation, with unchanged Metronidazoleaccounting for approximately 20% of the total. Both the parent compound and the hydroxylmetabolite possess in vitro antimicrobial activity.

Renal clearance of Metronidazole is approximately10 mL/min/1.73 m2. The average elimination half-life of Metronidazole in healthy subjects is eight hours.

1.1.2.4 Renal Impairment:

Decreased renal function does not alter the single-dose pharmacokinetics of Metronidazole. Subjects with end-stagerenal disease (ESRD; CLCR=8.1±9.1 mL/min) and who received a single intravenous infusion of Metronidazole 500 mg had no significant change in Metronidazole pharmacokinetics but had 2-fold higher Cmax of hydroxy-Metronidazole and 5-fold higher Cmax of Metronidazole acetate,compared to healthy subjects with normal renal function (CLCR=126±16 mL/min).Thus, on account of the potential accumulation of Metronidazole metabolites in ESRD patients, monitoring for Metronidazole associated adverse events is recommended.

1.1.2.5 Effect of Dialysis:

Following a single intravenous infusion or oral dose of Metronidazole 500mg, the clearance of Metronidazole was investigated in ESRD subjects undergoing hemodialysis or continuous ambulatory peritoneal dialysis (CAPD). A

hemodialysis session lasting for 4 to 8 hours removed 40% to 65% of the administered Metronidazole dose, depending on the type of dialyzer membrane used and the duration of the dialysis session. If the administration of Metronidazole cannot be separated from the dialysis session, supplementation of Metronidazole dose following hemodialysis should be considered. A peritoneal dialysis session lasting for 7.5 hours removed approximately 10% of the administered Metronidazole dose. No adjustment in Metronidazole dose is needed in ESRD patients undergoing CAPD.

1.1.2.6 Hepatic Impairment:

Following a single intravenous infusion of 500 mg Metronidazole, the mean AUC24 of Metronidazole was higher by 114% in patients with severe (Child-Pugh C) hepatic impairment, and by 54% and 53% in patients with mild (Child-Pugh A) and moderate (Child-Pugh B) hepatic impairment, respectively, compared to healthy control subjects. There were no significant changes in the AUC24 of hydroxyl-Metronidazolein these hepatically impaired patients. A reduction in Metronidazole dosage by 50% is recommended in patients with severe (Child-PughC)hepatic impairment. No dosage adjustment is needed for patients with mild to moderate hepatic impairment. Patients with mild to moderate hepatic impairment should be monitored for Metronidazole associated adverse events.

1.1.2.7 Geriatric Patients:

Following a single 500 mg oral or IV dose of Metronidazole, subjects >70 years old with no apparent renal or hepatic dysfunction had a 40% to 80% higher mean AUC of hydroxy-Metronidazole (active metabolite), with no apparent increase in the mean AUC of Metronidazole (parent compound), compared to young healthy controls <40 years old. In geriatric patients, monitoring for Metronidazole associated adverse events is recommended.

1.1.2.8 Pediatric Patients:

In one study, newborn infants appeared to demonstrate diminished capacity to eliminate Metronidazole. The elimination half-life, measured during the first 3 days of life, was inversely related to gestational age. In infants whose gestational ages were between 28 and 40 weeks, the corresponding elimination half-lives ranged from 109 to 22.5 hours.

1.1.2.9 Mechanism of Action:

Metronidazole exerts antibacterial effects in an anaerobic environment by the following possible mechanism: Once Metronidazole enters the organism, the drug is reduced by intracellular electron transport proteins. Because of this alteration to the Metronidazole molecule, a concentration gradient is created and maintained which promotes the drug's intracellular

transport. Presumably, free radicals are formed which, in turn, react with cellular components resulting in death of the bacteria.

Metronidazole is active against most obligate anaerobes, but does not possess any clinically relevant activity against facultative anaerobes or obligateaerobes.

1.1.3 Activity In Vitro and In Vivo:

Metronidazole has been shown to be active against most isolates of the following bacteria both in vitro and in clinical infections:

i) **Gram-positiveanaerobes**

Clostridiumspecies.

Eubacteriumspecies.

Peptococcusspecies.

Peptostreptococcus species.

ii) **Gram-negativeanaerobes**

Bacteroidesfragilis group (B. fragilis, B. distasonis, B. ovatus, B.hetaiotaomicron, B.vulgatus)and Fusobacterium species.

iii) **Protozoalparasites**

Entamoebahistolytica.

Trichomonasvaginalis.

The following in vitro data are available, but their clinical significance is unknown:

Metronidazole exhibits in vitro minimum inhibitory concentrations (MIC's) of <8 mcg/mL or less against most (≥ 90%) isolates of the following bacteria; however, the safety and effectiveness of Metronidazole in treating clinical infections due to these bacteria have not been established in adequate and well-controlled clinical trials.

i) **Gram-negativeanaerobes**

Bacteroidesfragilis group (B. caccae, B. uniformis)
Prevotella species (P. bivia, P. buccae, P. disiens)

1.1.4 Tablet pre-formulation:

Formulation is the process in which different chemical substances, including the active drug, are combined to produce a final medicinal product.

Pre-formulation involves the characterization of a drug's physical, chemical, and mechanical properties in order to choose what other ingredients (known asexcipients) should be used in the preparation. For example, in dealing with protein pre-formulation, an important aspect is to understand the solution behaviour of a given protein under a variety of stress conditions such as freezing, temperature, shear stress among others to identify mechanisms of degradation and therefore its mitigation.

Selection of certain type of an excipient for a selected drug formula should be done cautiously to give a proper quality of a formula.

1.1.5Stability studies:

The purpose of the stability study is to establish, based on testing and evaluating the stability information (including, as appropriate, results of the physical, chemical, biological, and microbiological test) a shelf life and label storage instructions applicable to all future batches of the drug product manufactured and packaged under similar circumstances.

In general, a drug substance should be evaluated under storage conditions (with appropriate tolerances) that test its thermal stability and, if applicable, its sensitivity to moisture. The storage conditions and the lengths of studies chosen were regarded as primary data which indicate to which extent the formulated tablets can withstand the conditions specified. So accordingly the stability study protocol was chosen as follows:

1- Accelerated condition stability
2- Real time condition stability

Data accumulated during the assessment period of the registration application should be submitted to the authorities if requested. Data from the accelerated storage condition and, if appropriate, from the intermediate storage condition can be used to evaluate the effect of short term excursions outside the label storage conditions. Long term, accelerated, and, where appropriate, intermediate storage conditions for drug substances are detailed in the sections below. The general case applies if

the drug substance is not specifically covered by a subsequent section. Alternative storage conditions can be used if justified. table 3 below shows the prescribed stability conditions:

Table: The prescribed stability conditions:

Study	Storage conditions
Long term	25+_ 2 C/different range of humidity
Accelerated conditions	40+_2/75+_5

The data may show so little degradation and so little variability that it is apparent from looking at the data that the requested retest period will be granted. Under these circumstances, it is normally unnecessary to go through the formal statistical analysis; providing a justification for the omission should be sufficient. the stability study carried here is only for conformation to which extent this changes in the formula will affect the stability and physical and chemical properties of the finished product.

The analytical work performed was as to follow the full monograph (BP) and to apply all In- process checks and finished product analysis as described in the chapter three procedures and methodology. The monograph was choosed because the analytical methods used for such a comparative study andfor testing the stability indicating parametersshould be validated to

give the least possible errors so as to provide the evidence that the only conclusion is because of the formulation itself.

1.2 Pharmaceutical Analysis:

Pharmaceutical Analysis may be defined as the application of analytical procedures used to determine the purity, safety and quality of drugs and chemicals. The term “Pharmaceutical analysis” is otherwise called quantitative pharmaceutical chemistry. Pharmaceutical analysis includes both qualitative and quantitative analysis of drugs and pharmaceutical substances starts from bulk drugs to the finished dosage forms. In the modern practice of medicine, the analytical methods are used in the analysis of chemical constituents found in human body whose altered concentrations during disease states serve as diagnostic aids and also used to analyze the medical agents and their metabolites found in biological system.

Qualitative inorganic analysis seeks to establish the presence of given element or inorganic compound in a sample.

Qualitative organic analysis seeks to establish the presence of a given functional group or organic compound in a sample.

Quantitative:

Quantitative analysis seeks to establish the amount of a given element or compound in a sample.

The term “quality” as applied to a drug product has been defined as the sum of all factors, which contribute directly or indirectly to the safety, effectiveness and reliability of the product. These properties are built into drug products through research and during process by procedures collectively referred to as “Quality control”. Quality control guarantees within reasonable limits that a drug products:

- Is free of impurities.
- Is physically and chemically stable
- Contains the amount of active ingredients as stated on the label.
- Provides optimal release of active ingredients when the product is administered.

Most modern analytical chemistry is categorized by two different approaches such as analytical targets or analytical methods:

- ✓ By Analytical Targets.
- ✓ By Analytical Methods.
- ✓ Bioanalytical chemistry.
- ✓ Material analysis.
- ✓ Chemical analysis.
- ✓ Environmental analysis.
- ✓ Forensics.
- ✓ Mass spectrometry.
- ✓ Spectrophotometry.
- ✓ Colorimetry.
- ✓ Chromatography.
- ✓ Electrophoresis.

- ✓ Crystallography.
- ✓ Microscopy.
- ✓ Electrochemistry.
- ✓ Thermal methods.

Pharmaceutical analysis provides information on the identity, purity, content and stability of starting materials, excipients and active pharmaceutical ingredients (APIs). A distinction is made between analysis of the pure active ingredients used to cure, alleviate, prevent or identify ill-nesses and diseases (active ingredient analysis) and analysis of medicinal preparations. The latter can exist in various forms (ointments, tinctures, pills, lotions, suppo- sitories, infusions, drops, etc.) and consists of the phar-maceutically active substance and at least one pharma-ceutical excipient. Impurities usually stem from the syn- thesis of the active ingredient; they are usually monitored according to the guidelines of the ICH (InternationalConference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) and the pharmacopoeias. [1]

Assay:[2]

An assay is an investigative (analytic) procedure in laboratory medicine, pharmacology, environmental biology, continuous delivery, and molecular biology for qualitatively assessing or quantitatively measuring the presence or amount or the functional activity of a target entity (the analyte). The analyte can be a drug or biochemical substance or a cell in an organism or

organic sample.[1][2] The measured entity is generally called the analyte, or the measurand or the target of the assay. The assay usually aims to measure an intensive property of the analyte and express it in the relevant measurement unit (e.g. molarity, density, functional activity in enzyme international units, degree of some effect in comparison to a standard, etc.).

If the assay involves addition of exogenous reactants (the reagents) then their quantities are kept fixed (or in excess) so that the quantity (and quality) of the target is the only limiting factor for the reaction/assay process, and the difference in the assay outcome is used to deduce the unknown quality or quantity of the target in question. Some assays (e.g., biochemical assays) may be similar to or have overlap with chemical analysis and titration. But generally, assays involve biological material or phenomena which tend to be intrinsically more complex either in composition or in behavior or both. Thus reading of an assay may be quite noisy and may involve greater difficulties in interpretation than an accurate chemical titration. On the other hand, older generation qualitative assays, especially bioassays, may be much more gross and less quantitative (e.g., counting death or dysfunction of an organism or cells in a population, or some descriptive change in some body part of a group of animals).

Assays have become a routine part of modern medical, environmental, pharmaceutical, forensic and many other businesses at various scales from industrial to curbside or field level. Those assays that are very highly commercially demanded have been well investigated in research and development sectors of professional industries, undergone generations of development and sophistication, and become copyrighted intellectual properties via highly competitive process patenting. Such industrial scale assays as these are often done in well equippedlaboratories and with automated organization of the procedure—from ordering an assay to pre-analytic sample processing (sample collection, necessary manipulations e.g. spinning for separation or other processes, aliquoting if necessary, storage, retrieval, pipetting/aspiration etc.). Analytes are generally tested in high throughput AutoAnalyzers, and the results are verified and automatically returned to ordering service providers and end users. These are made possible through use of advanced Laboratory informatics system that interfaces with multiple computer terminals with end users, central servers, the physical autoanalyser instruments, and other automata.

General steps of any assay: [3]

An assay (analysis) is never an isolated process and must be preceded by pre- and post analytic procedures. The information communication (e.g. request to perform an assay and further

information processing) or specimen handling (e.g. collection transport and processing) that are done until the beginning of an assay are the preanalytic steps. Similarly, after the assay, the result may be documented, verified and transmitted/communicated in steps that are called post-analytic steps. Like any multistep information handling and transmission systems, variation and errors in the communicated final results of an assay involves corresponding parts in every such step i.e. not only analytic variations and errors intrinsic to the assay itself but also variations and errors involved in preanalytic and post analytic steps. Since the assay itself (the analytic step) gets a lot of attention, steps that get less attention by the chain of users i.e. the preanalytic and the post analytic steps are often less stringently regulated and generally more prone to errors- e.g. preanalytic steps in medical laboratory assays may contribute to 32-75% of all lab errors.

Assays can be very diverse, but generally involve the following general steps:

1. **Sample processing/manipulation** in order to selectively present that target in a discernible/measurable form to a discrimination/identification/detection system. It might involve a simple centrifugal separation or washing or filtration or capture by some form of selective binding or it may even involve modifying the target e.g. epitope retrieval

in immunological assays or cutting down the target into pieces e.g. in Mass Spectrometry. Generally there are multiple separate steps done before an assay and are called preanalytic processing. But some of the manipulations may be inseparable part of the assay itself and will not thus be considered pre-analytic.

2. **Target specific DISCRIMINATION/IDENTIFICATION principle**: to discriminate from background (noise) of similar components and specifically identify a particular target component ("analyte") in a biological material by its specific attributes. (e.g. in a PCR assay a specific oligonucleotide primer identifies the target by base pairing based on the specific nucleotide sequence unique to the target).
3. **Signal (or target) AMPLIFICATION System**: The presence and quantity of that analyte is converted into a detectable signal generally involving some method of signal amplification, so that it can be easily discriminated from noise and measured - e.g. in a PCR assay among a mixture of DNA sequences only the specific target is amplified into millions of copies by a DNA polymerase enzyme so that it can be discerned as a more prominent component compared to any other potential components. Sometimes the concentration of the analyte is too large and in that case the assay may involve sample dilution or some sort of signal diminution system which is a negative amplification.

4. **Signal DETECTION (and interpretation) system**: A system of deciphering the amplified signal into an interpretable output that can be quantitative or qualitative. It can be visual or manual very crude methods or can be very sophisticated electronic digital or analog detectors.
5. **Signal enhancement and noise filtering**: may be done at any/all of the steps above. Since the more downstream a step/process during an assay, the higher the chance of carrying over noise from the previous process and amplifying it, multiple steps in a sophisticated assay might involve various means of signal-specific sharpening/enhancement arrangements and noise reduction or filtering arrangements. These may simply be in the form of a narrow band-pass optical filer, or a blocking reagent in a binding reaction that prevents nonspecific binding or a quenching reagent in a fluorescence detection system that prevents "autofluorescence" of background objects.

Assay types based on the nature of the assay process:

Depending on whether an assay just looks at a single time point or timed readings taken at multiple time points, an assay may be:

1. **End point assay**: when the only reading that matters is the end result after a fixed assay incubation period.

2. **Kinetic assay**: when readings are taken multiple times at fixed time intervals during an assay and a kinetic graph of the readings is important.

Depending on how many targets or analytes are being measured:

1. Usual assay are simple or single target assays which is usually the default unless it is called multiplex.
2. Multiplex assays are assays that in a same reaction detect multiple analytes simultaneously.

Depending on the quality of the result produced, assays may be classified into:

1. **Qualitative assay**, i.e. assays which generally give just a pass or fail, or positive or negative or some such sort of only small number of qualitative gradation rather than an exact quantity.
2. **Semi-quantitative assays**, i.e. assays that give the read-out in an approximate fashion rather than an exact number for the quantity of the substance. Generally they have a few more gradations than just two outcomes, positive or negative, e.g. scoring on a scale of 1+ to 4+ as used for blood grouping tests based on RBC agglutination in response to grouping reagents (antibody against blood group antigens).

3. **Quantitative assays**, i.e. assays that give accurate and exact numeric quantitative measure of the amount of a substance in a sample. An example of such an assay used in coagulation testing laboratories for the commonest inherited bleeding disease - Von Willebrand disease is VWF antigen assay where the amount of VWF present in a blood sample is measured by an immunoassay.
4. **Functional assay**, i.e. an assay that tries to quantify functioning of an active substance rather than just its quantity. The functional counterpart of the VWF antigen assay is Ristocetin Cofactor assay, which measures the functional activity of the VWF present in a patients plasma by adding exogenous formalin-fixedplatelets and gradually increasing quantities of drug named ristocetin while measuring agglutination of the fixed platelets. A similar assay but used for a different purpose is called Ristocetin Induced Platelet Aggregation or RIPA, which tests response of endogenous live platelets from a patient in response to Ristocetin (exogenous) & VWF (usually endogenous).

Depending on the general substrate on which the assay principle is applied:

1. **Bioassay**: when the response is biological activity of live objects e.g.
    1. Organism (e.g. mouse injected with a drug)

2. ex vivo body part (e.g. leg of a frog)
3. ex vivo organ (e.g. heart of a dog)
4. ex vivo part of an organ (e.g. a segment of an intestine).
5. tissue (e.g. limulus lysate)
6. cell (e.g. platelets)

2. **Ligand binding assay** when a ligand (usually a small molecule) binds a receptor (usually a large protein).
3. **Immunoassay** when the response is an antigen antibody binding type reaction.

Depending on the nature of the signal amplification system assays may be of numerous types, to name a few:

1. **Enzyme activity assay**: Enzymes may be tested by their highly repeating activity on a large number of substrates when loss of a substrate or the making of a product may have a measurable attribute like color or absorbance at a particular wavelength or light or chemiluminiscence or electrical/redox activity.
2. Light detection systems that may use amplification e.g. by a photodiode or a photomultiplier tube or a cooled charge coupled device.
3. Radioisotope labeled substrates as used in radioimmunoassays and equilibrium dialysis assays and

can be detected by the amplification in GM counters or X-ray plates, or phosphorimager

4. Polymerase Chain Reaction Assays that amplifies a DNA (or RNA) target itself rather than the signal

Assays may be a combination of the above e.g. enzyme Immuno assay or EIA, enzyme linked immunosorbent assay.

Depending on the nature of the Detection system assays can be based on:

1. **Colony forming** or virtual colony count: e.g. by multiplying bacteria or proliferating cells.
2. **Photometry** / **spectrophotometry** When the absorbance of a specific wavelength of light while passing through a fixed path-length through a cuvette of liquid test sample is measured and the absorbance is compared with a blank and standards with graded amounts of the target compound. If the emitted light is of a specific visible wavelength it may be called **colorimetry**, or it may involve specific wavelength of light e.g. by use of laser and emission of fluorescent signals of another specific wavelength which is detected via very specific wavelength optical filters.
3. **Transmittance** of light may be used to measure e.g. clearing of opacity of a liquid created by suspended

particles due to decrease in number of clumps during a platelet agglutination reaction.

4. **Turbidimetry** when the opacity of straight-transmitted light passing through a liquid sample are measured by detectors placed straight across the light source.
5. **Nephelometry** when the scattered lights are measured by detectors placed at fixed angles to the path of light.
6. **Reflectometry** When color of light reflected from a (usually dry) sample or reactant is assessed e.g. the automated readings of the strip urine dipstick assays.
7. Viscoelastic measurements e.g. viscometry, elastography (e.g. thromboelastography)
8. Counting assays: e.g. optic Flowcytometric cell or particle counters, or coulter/impedance principle based cell counters
9. Imaging assays, that involve image analysis manually or by software:
    1. **Cytometry**: When the size statistics of cells is assessed by an image processor.
10. Electric detection e.g. involving amperometry, voltametry, coulometry may be used directly or indirectly for many types of quantitative measurements.
11. Other physical property based assays may use
    1. Osmometer
    2. Viscometer
    3. Ion Selective electrodes

1.2.2 Stability - Indicating Methodologies:[1]

Analytical methodologies that are specific to the major analyte that are also capableof separating and quantifying potential degradation products and impurities, whilesimultaneously maintaining specificity and ccuracy are deemed stability indicating.

Traditional stability - indicating high - performance liquidchromatography (HPLC)methodologies for small molecules are developed and validated with relative ease.

Typically, the stability - indicating nature of an analytical method can be demonstratedby subjecting the product to forced degradation in the presence of heat, acid,alkali, light, or peroxide. If degradation products are sufficiently well resolvedfrom the active while maintaining specificity and curacy, the method is suitable.

In contrast to small molecules, there is no one “gold standard” analytical methodologythat can be utilized to determine the potential degradation products and impuritiesin the milieu that may constitute a biopharmaceutical drug product. Furthermore,a one - dimensional structure assessment (e.g., in terms of an absorption spectrum)does not give any indication of the overall activity of the product, as is the case withtraditional small molecules. Thus, the stability assessment of biopharmaceuticals willtypically comprise a multitude of methodologies that when taken together give anindication of the stability of the product.

The overall goal is to assess the structuralelements of the compound as well as attempt to determine the relative quantitiesof potential degradation products, as well as product isoforms and impurities thatare inherent to the expression systems utilized for API manufacture. However, it isstill advised that bioactivity determinations are made at appropriate intervalsthroughout the stability program, as discussed below. Furthermore, any biopharmaceutical stability program should also minimally include an evaluation of the in vitro immunogenicity profile of the product with respect to time, temperature, and otherpotentialdegradative conditions.

STORAGE

Metronidazole Oral Suspension should be protected form light .

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of metronidazole

Chapter Two

2. Objective of Research

The objective of this research is to evaluate the stability of Metronidazole Active Pharmaceutical Ingredients; which are: assa

1. DircetSunlight condition.
2. Room .Temp
3. 50

Chapter Three

4. Theory

Procedures and Methodology

Acidity

pH , 500 to 6.5 Appendix VL

Metronidazole

Carry out the method for liguid chromatography , Appendix IIID , using the following solutions for solution (1) mix a quantity of the oral suspension containing the equivalent of 200 mg of metronidazoale with 150 ml of methanol , add sufficient water , with mixing and cooling , to produce 250 ml . mix and centrifuge . For solution (2) dissolve20 mg of metronidazoale 205 ml . Dilute 1 volume to 10 volumes with methanol (60%) .

The chromatographic procedure may be carried out using (a)a stainless steel column (25 cm solution (1) the area any peak corresponding to metronidazole is x 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5um) (Spheris orb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1.0 ml per minute a mixture of volumes of 1.25 % w/v colution of ammonium acetate , adjusted to pH 7.0 with dilute acetic acid or dilute ammonia R 1 , as appropriate , and 60 volumes of methanol and (c) a detection wavelength of 310 nm .

In the chromatogram obtained with not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1 %) .

Method of analysis

Carry out the method for liquid chromatography , Appendix IIID , using the following solutions . solution (1) mix a quantity of the oral suspension the equivalent of 200 mg of metronidazole with 150 ml of methanol , add sufficient with mixing and cooling to produce 250ml , mix and centrifuge Dilute

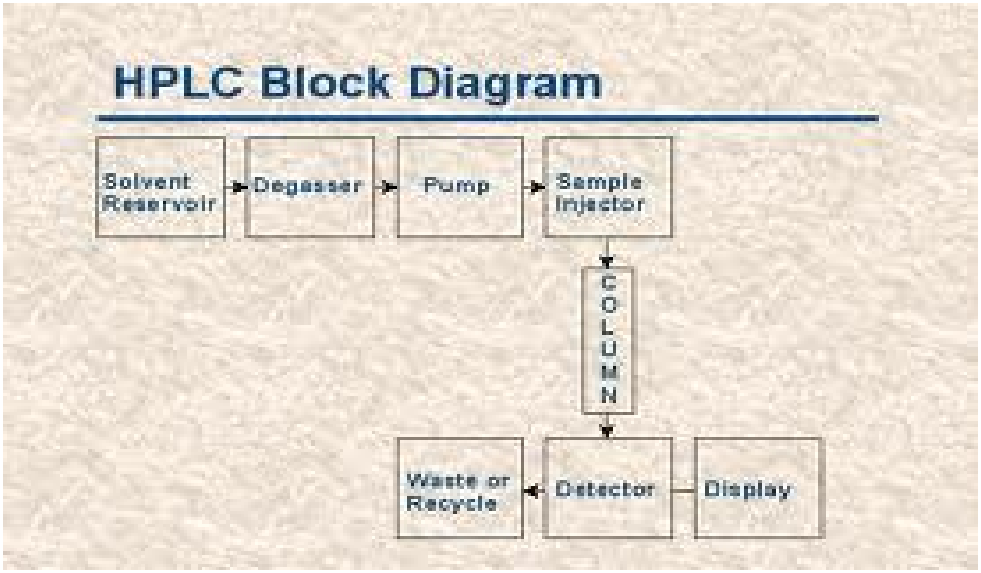
1 volume to 10 volumes with methanol(60%) For solution (2) dissolve 62.5 mg of metronidazole benzoate BPCRS in 1 ml of dimethylformamide and 30 ml . dilute 1 volume to 10volumes with methanol (60%)

Determine the weight per of the oral suspension . Appendix VG , and calculate benzoate BPCRS . the content of C_6 H_9 N_3 O_3 weight in volume , using the declared content of C_6 H_9 N_3 O_3 in metronidazole

Instrumentation and Apparatus

Hardware of HPLC:

The schematic of an HPLC instrument typically includes a sampler, pumps, and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components. A digital microprocessor and user software control the HPLC instrument and provide data analysis. Some models of mechanical pumps in a HPLC instrument can mix multiple solvents together in ratios changing in time, generating a composition gradient in the mobile phase. Various detectors are in common use, such as UV/Vis, photodiode array (PDA) or based on mass spectrometry. Most HPLC instruments also have a column oven that allows for adjusting the temperature the separation is performed at.



2.4 Application of HPLC:

HPLC is optimum for the separation of chemical and biological compounds that are non volatile.

Typical non-volatile compounds are: [19]

- Pharmaceuticals like aspirin, ibuprofen, or acetaminophen (Tylenol).
- Salts like sodium chloride and potassium phosphate.
- Proteins like egg white or blood protein.
- Organic chemicals like polymers (e.g. polystyrene, polyethylene).
- Heavy hydrocarbons like asphalt or motor oil.
- Many natural products such as ginseng, herbal medicines, plant extracts.
- Thermally unstable compounds such as trinitrotoluene (TNT), enzymes.

HPLC has been used for medical (e.g. detecting vitamin D levels in blood serum), legal (e.g. detecting performance enhancement drugs in urine), research (e.g. separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and manufacturing (e.g. during the production process of pharmaceutical and biological products) purposes.

3.1 High Performance Liquid Chromatography:

3.1.1 Introduction:

High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography), is a technique in analytic chemistry used to separate the components in a mixture, to identify each component, and to quantify each component. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column.

HPLC has been used for medical (e.g. detecting vitamin D levels in blood serum), legal (e.g. detecting performance enhancement drugs in urine), research (e.g. separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and manufacturing (e.g. during the production process of pharmaceutical and biological products) purposes.[1]

Chromatography can be described as a mass transfer process involving adsorption. HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with a sorbent, leading to the separation of the sample components. The active component of the column, the sorbent, is typically a granular material made of solid particles (e.g. silica, polymers, etc.), 2–50 micrometers in size. The components of the sample mixture are separated from each other due to their different degrees of interaction with the sorbent particles. The pressurized liquid is typically a mixture of solvents (e.g. water, acetonitrile and/or methanol)

and is referred to as a "mobile phase". Its composition and temperature play a major role in the separation process by influencing the interactions taking place between sample components and sorbent. These interactions are physical in nature, such as hydrophobic (dispersive), dipole–dipole and ionic, most often a combination thereof.

Reagents and chemicals:

Metronidazole sample
Methanol (60%)
Distteled water
Metronidazole benzoate
Ammonium acetate
Acetic acid
Dimethyl formamide

Appratus:

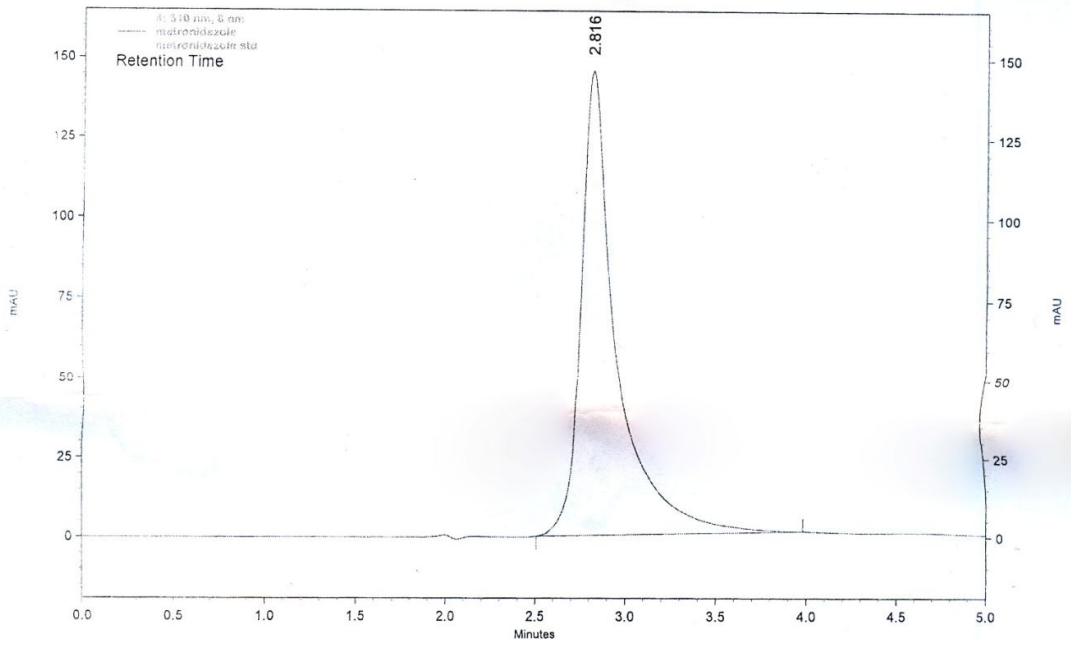
Centrifuge
HPLC
PH meter
Sencilire Balance
Valuemetric flask
Beakers
Pipette
Measuring sylinder

Chapter Four

Results

Shimadzu CLASS-VP V6.14 SP1
Area % Report Page 1 of 2

Method Name: C:\CLASS-VP\Methods\meteronidazole.met
Data Name: C:\CLASS-VP\metronidazole std
User: System
Acquired: 6/19/2014 1:59:21 PM
Printed: **6/22/2014 11:16:11 AM**
injection volume 10 µL



4: 310 nm, 8 nm

Name	Retention Time	Area	Height
Meteronidazole	2.816	2020671	145838

Shimadzu CLASS-VP V6.14 SP1
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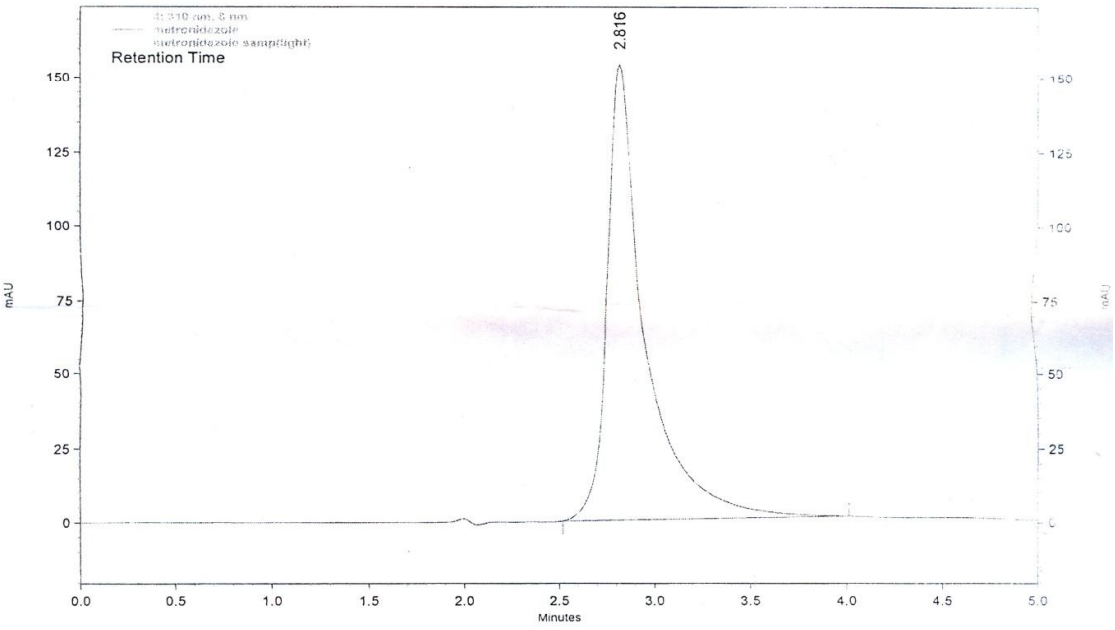
Method Name: C:\CLASS-VP\Methods\meteronidazole.met
Data Name: C:\CLASS-VP\metronidazole samp(light)
User: System
Acquired: 6/19/2014 2:37:03 PM
Printed: 6/22/2014 11:53:52 AM
injection volume 10



4: 310 nm, 8 nm

Name	Retention Time	Area	Height
Meteronidazole	2.816	2140597	153835

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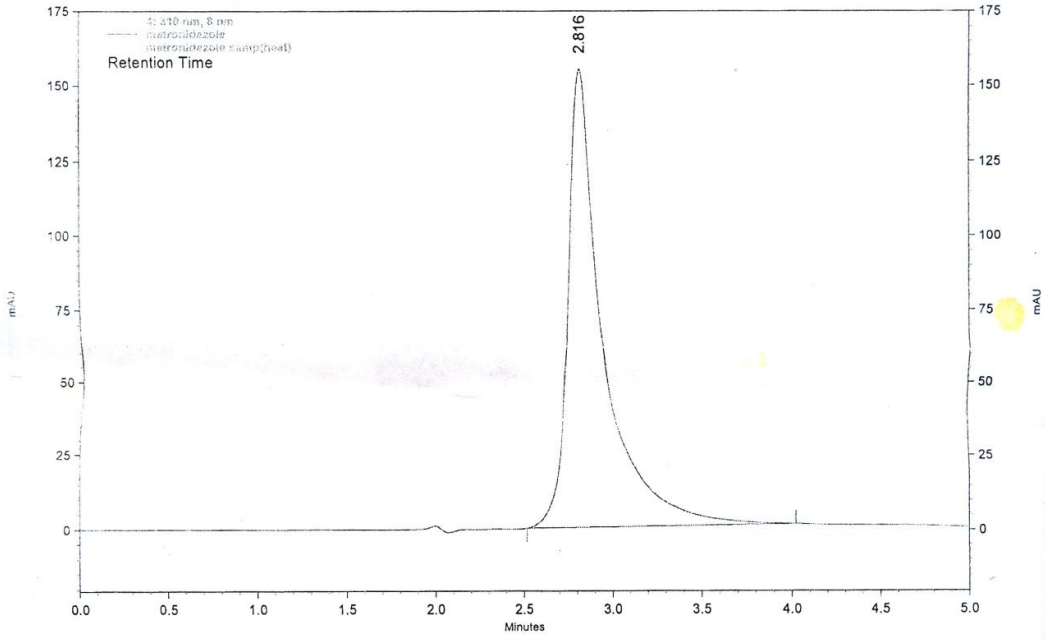
Method Name: **C:\CLASS-VP\Methods\meteronidazole.met**
Data Name: C:\CLASS-VP\metronidazole samp(heat)
User: System
Acquired: 6/19/2014 2:29:15 PM
Printed: 6/22/2014 11:53:02 AM
injection volume 10



4: 310 nm, 8 nm

Name	Retention Time	Area	Height
Meteronidazole	2.816	2137443	154628

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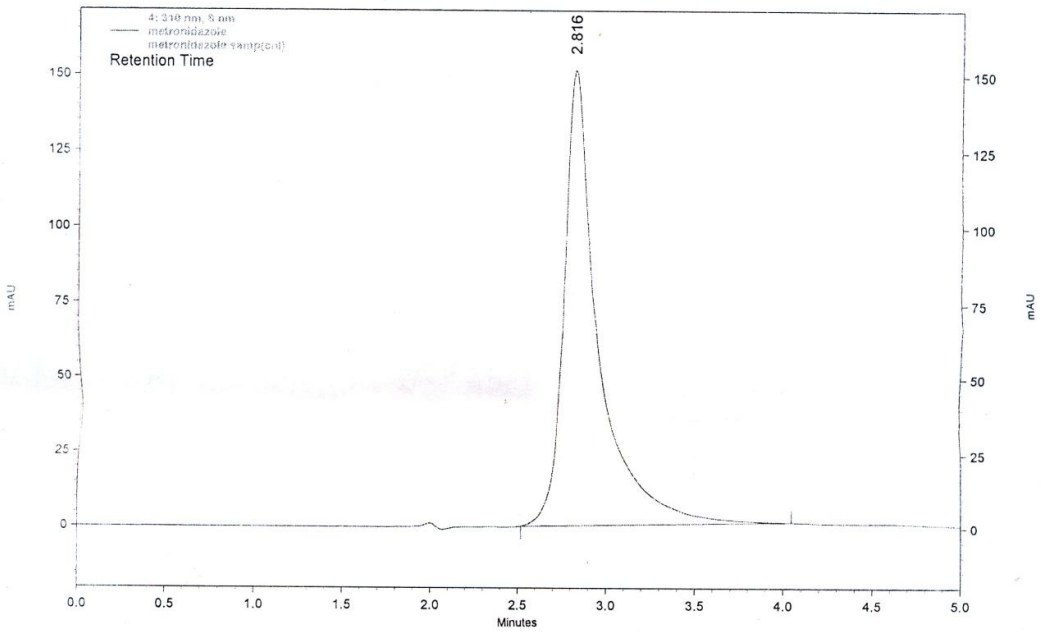
Method Name: **C:\CLASS-VP\Methods\meteronidazole.met**
Data Name: C:\CLASS-VP\metronidazole samp(cnl)
User: System
Acquired: 6/19/2014 2:21:33 PM
Printed: **6/22/2014 11:47:59 AM**
injection volume 10



4: 310 nm, 8 nm

Name	Retention Time	Area	Height
Meteronidazole	2.816	2093373	151227

Calculation

Sample(1)

Volume of sample 5ml

Weight of STD321.2metronidazole benzoate

Area under curve of sample = 2093373

Area under curve of standerd =2020671

Content = Area under curve of sample/Area under curve of standard x100%

Meteronidazole sample(cnl)=2093373/2020671x100=103.59792

Sample (2)

Volume of sample 5ml

Weight of STD321.2metronidazole benzoate

Area under curve of sample = 2137443

Area under curve of standerd =2020671

Content = Area under curve of sample/Area under curve of standard x100%

Meteronidazole sample(heat)=2137443/2020671x100=105.77888

Sample (3)

Volume of sample 5ml

Weight of STD321.2metronidazole benzoate

Area under curve of sample = 2140597

Area under curve of stander =2020671

Content = Area under curve of sample/Area under curve of standard x100%

]Meteronidazole
sample(light)=2140597/2020671x100=105.93496

Chapter 5

Sample (1)

. solution (1) mix a quantity of the oral suspension the equivalent of 200 mg of metronidazole with 150 ml of methanol ,and add sufficient with mixing and cooling to produce 250ml , mix and centrifuge Diluted 1 volume to 10 volumes with methanol(60%) For solution (2) dissolve 62.5 mg of metronidazole benzoate BPCRS in 1 ml of dimethylformamide and 30 ml . dilute 1 volume to 10volumes with methanol (60%)

The Result area under curve of sample (1) is 2093373

The result rea under curve of the standerd is 2020671

Sample (2)

solution (1) mix a quantity of the oral suspension the equivalent of 200 mg of metronidazole with 150 ml of methanol ,and add sufficient with mixing and cooling to produce 250ml , mix and centrifuge Diluted 1 volume to 10 volumes with methanol(60%) For solution (2) dissolve 62.5 mg of metronidazole benzoate BPCRS in 1 ml of dimethylformamide and 30 ml . dilute 1 volume to 10volumes with methanol (60%)

The Result area under curve of sample (2) is 2137443

The result rea under curve of the standerd is 2020671

Sample (3)

solution (1) mix a quantity of the oral suspension the equivalent of 200 mg of metronidazole with 150 ml of methanol ,and add sufficient with mixing and cooling to produce 250ml , mix and centrifuge Diluted 1 volume to 10 volumes with methanol(60%) For solution (2) dissolve 62.5 mg of metronidazole benzoate BPCRS in 1 ml of dimethylformamide and 30 ml . dilute 1 volume to 10volumes with methanol (60%)

The Result area under curve of sample (3) is 2140597

The result area under curve of the standerd is 2020671

Conclusion

The drug (metronidazole suspension) is stable when exposed to direct sun light for 5 days and also it is stable when exposed to 50 for 5 days