Sudan University of Science and Technology

College of Sciences

Department of **Chemistry**

WHO stability Protocol of Drugs

(SalbutamolSulphate)

A thesis Submitted in Partial Fulfillment for the Requirements of a Bachelor Degree in Chemistry

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الآية

قال تعالى:

بسم الله الرحمن الرحيم

اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ ۚ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ ۖ الْمِصْبَاحُ فِي زُجَاجَةٍ ۖ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِن شَجَرَةٍ مُّبَارَكَةٍ زَيْتُونَةٍ لَّا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ ۚ نُّورٌ عَلَىٰ نُورٍ ۗ يَهْدِي اللَّهُ لِنُورِهِ مَن يَشَاءُ ۚ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ ۗ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ ﴿﴾

صدق الله العظيم

سورة النور، الآية (35)

I

Dedication

To those people who mean something to me...

To those who have touched my life in one way or another...

To those who make me smile when I really need it....

To those that make me see the brighter side when I am really down...

To those who I want to let them know that I appreciate their love and support...

My Dear Father, Mother...

To All of my family...

My sisters, Uncles, Aunts...

To My friends whom I have non-forgettable moments with them...

Acknowledgement

Abstract:

Salbutamol is apharmaceutical drug for the relief of bronchospasm in condition such as asthma.It is sold as racemic mixture in its forms (S)and(R).In this present work 1.3g from sample was crushed and dissolved in amixture of ammonium acetate dissolved in filter distilled water,2-propanol then adjusted PH of 4.5 by adding glacial acetic acid then shaked for 90minute and separation occurred using automatic HPLC.

Identifications of salbutamol were carried out using IR and UV spectroscopy using twobuffers of solutions, phosphate buffer, sodium hydroxide buffer.

In UV data the real λ max were appeared in PH 9and10 due to the amino group and phenolic group respectively.

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ChapterOne

Introduction

WHO STABILITY PROTOCOL OF DRUGS

1.1 Introduction:-

Stability – Theoritical consideration

1.1.1 Definition:

The capacity of a drug or product to remain within established specifications of identifies, quality, purity in a specific period of time.

1.1.1.1 United State Pharmacopeia:

Defines stability of pharmaceutical product as extent to which a product retains with in specified limits and throughout it period of storage and use (ie shelf life).

1.1.1.2 Shelf life:

It is defined as the time required for the concentration of the reactant to reduce to 90% of its initial concentration. Represented as t 90 and the unit of time/conc.

$$t\,90 = \frac{(a - 0.9\,a)}{k0} = \frac{0.1\,a}{k0}$$

Where   a = initial concentration.

K_0 = specific rate constant for zero order reaction.

1.2 Factors effecting drug stability:

1.2.1 The primary factors effecting stability:

pH, Temperature, Moisture, humidity, light, storage closure, and containers, oxygen.

The major factors effecting drug stability are:

Particle size (suspension and emulsion), pH, additives and molecular binding and diffusion of drugs and **excipients**.

1.3 Objectives:

- To determine maximum expiration date/shelf life.
- To provide better storage condition.
- To determine the packaging components.
- Togather information during performulation stage to produce astable product.

1.4 Type of stability:

1- Chemical stability.
2- Physical stability.
3- Microbiologic stability.
4- Thearapeutical stability.
5- Toxicologic stability.

1.4.1 Types of stability that must be considered for any drug:

▪ **Chemical:**

Each active ingredient retains its chemical integrity and labeled potency with in the specified limit.

▪ **Physical:**

The physical stability properties includes appearance, palatability, uniformty, dissolution and suspendability are retained.

▪ **Microbiological:**

Sterility or resistance to microbial growth is retained according to specified requirement.

- **Therapeutic:**

Therapeutic activity remains unchanged.

- **Toxicologic:**

No significant increase in toxicity occurs.

1.5 Relative Humidity:

- Relative humidity is the ratio of the partial pressure of water vapor in an air water mixture to the saturated vapor pressure of water at prescribed temperature .
- Relative humidity depends on temperature and pressure.

1.6 Regulatory Requirements:

Stability study requirement and expiration dates are covered in the current GMP USP and FDA.

- GMP (Good Manufacturing Practice) states that there will be written testing program design to access the stability characteristics of drug products. And result of such stability testing will be used to determine appropriate storage condition and expiration dates.

1.7 Stability studies for pharmaceutical products:

- Stable tablets retain their original size, shape, weight, roughness, colour variation, cracking under normal handling and storage conditions throughout their shelf life.
- **Friability test:**   studies revel the physical instability if any in tablet.maximum weight eight loss should not be more than 1%.
- **Hardness test:** shows resistance to crushing.

- **Color stability**: by colorimeter, reflectometer with heat ,sun light and intense artificial light.
- Uniformity of weight, odor, texture, drug and moisture content, humidity effects are also studied during a tablet test.

1.8 Gelatine Capsule:

- Gelatine capsules are found to be stable in dry conditions but they rapidly reach equilibrium with the atmospheric conditions under they are stored.
- This showsgelatin capsulesare largely effected by temperature and humidity and susceptibility to microbial degradation
- Soft gelatin capsule have relative humidity 20 to 30% at 21 to 24C.
- Hard gelatin capsule contain 13 to 16% moisture.
- Humidity – capsule shell softens and becomes sticky.
- Dried capsule shell becomes brittle and crack.
- Hard gelatin capsule are tested for brittleness, dissolution, water content and level of microbial contamination.
- **Emulsion**s: Tested for phase separation, pH, viscosity, level of microbial contamination and distribution of dispersed globules.
- **Oral Solutions and Suspensions:**

Formation of precipitate, clarity for solutions, PH, viscosity, microbial contamination.

- **Nasal Sprays, Solution and Suspensions:**

Clarity (for solution), level of microbial contamination, pH , particulate matter, unit spray medication, content uniformity, droplet and/or particle size distribution, weight loss, pump delivery.

Topical, Opthalmic and Otic Preparation:

Included in this broad category are ointments, creams, lotions, paste, gel, solutions, eye drops.

- **Topical:**

Preparations should be evaluated for clarity homogeneity, PH, resuspendibility for lotions, consistency, viscosity, particle size distribution, level of microbial contamination/ sterility and weight loss.

- **Topical For Ophthalmic or OticPrepration:**

Should include the following additional attributes: sterility particulate matter and extractable .

Suppositories:

Softening range, dissolution (at 37 C)

Parenterals:

Color, clarity (for solutions), particulate matter, PH, sterility pyogen/ endotoxins. stability studies for powders for injection solution, include color monitoring. reconstitution time and water content.

1.9 Degradative Pathways of Pharmaceutical Dosage Forms:

- Degradation of active drug leads to lowering of quantity of the therapeutic agent in the dosage from.
- It may not be extensive, a toxic product formation may take place due to decomposition instability of drug product can lead to adecrease the bioavailability.
- Changes in physical appearance of give dosage form may take place.

- Degradation may increase or may decrease the potency of drug.

Sometimes active drug may retain its potency ,but excipients   like - antimicrobial, preservatives, solubilizers, emulsifying   agent may degrade, lead to compromising the integrity of drug product.

Example:

Drugs   like   5- fluorouracil, carbamazipine, digioxin   and theophylline have narrow therapeutic indices these needs to be carefully treated in patient so that plasma levels are neither too high as to be toxic nor too low as to be ineffective

1.9.1 Degradation May be of Two Types:

- Physical Degradation

- Chemical Degradation

* Oxidation

* Decarboxylation

* Photolysis

* Racemization

* Hydrolysis

1.9.1.1 Physical Degradation:

The physical stability properties includes appearance, palatability, uniformity, dissolution and suspend ability are retained. Maintained throughout the shelf life of drug.

It includes following:

*Loss of water.

*Loss of volatile oil.

*Water absorbance.

*Polymorphism.

*Color change.

Physical Degradation includes Following:

Loss of volatile content:

Volatile compounds used such as:

-Alcohol ether, camphor oils, etc. Try to escape from the formulation leads to degradation.

*Loss of water:

-Water Loss from liquid preparation (o\w emulsion) lead to changes instability.

-It causes crystallization of drug product . Which may lead to increase in potency and decrease in weight.

*Water absorbance:

Pharmaceutical formulations which are hygroscopic in nature absorb the water form its external environment leads to degradation.

*Polymorphism:

Astable crystal form is effected leads to the formation of polymorph and cause in stability in formulation.

*Color Change:

Loss or development of color may occur.

1.9.1.2 Chemical degradation:

Chemical degradation of a dosage from occurs through several pathways like- hydrolysis., oxidation, decarboxylation, photo-lysis, racemization. Which may lead to lowering of therapeutic agent in the dosage form , formation of toxic product, decreased bioavailability etc.

- **Hydrolysis**:

- Main classes of drugs that undergo hydrolysis are the ESTER,AMIDE,ALKALI,ACID.

*Ester hydrolysis:

Involve acyl-acid cleavage.

Example of drugs: aspirin, atropine, physostigmine ,procaine.

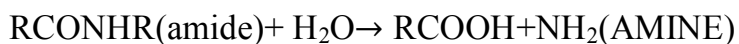R. COOR(ester) + $H_2O \rightarrow$ RCOOH(acid)+HOR(alcohol).

*Amide hydrolysis

* Is more stable than ester, susceptible to specific and general acid base hydrolysis.

It involves cleavage of amide linkage to give an amine instead of alcohol as in case of esters.

Example of drugs: Chloramphenicol, barbiturates.

RCONHR(amide)+ $H_2O \rightarrow$ RCOOH+NH_2(AMINE)

1.10 Protection against Hydrolysis:

- Avoiding contact with moisture at time of manufacture.
- Packaging in suitable moisture resistant packs such as strip packs.
- In liquid dosage form since, hydrolysis is acid or base catalyzed, an optimum pH for max stability should be selected and the formulation should be stabilized at this pH by inclusion of proper buffering agents.
- Hydrolysis of certain drugs such as benzocaine and procaine can be decreased by the addition of specific complexing agent like caffeine to the drug solutions.
- Hydrolysis susceptible drug such as penicillin and derivatives can be prevented by formulating them in the dry powder from for reconstitution.

Oxidation:

- Oxidation is controlled by environment ie light, trace elements.
- Occurs when exposed to atmospheric oxygen.
- Either the addition of oxygen or removed of hydrogen.
- Oxidation is the loss of electrons while reduction is the gain of electrons.

Auto oxidation:

-The reaction between the compounds and molecular oxygen is required for initiating the chain reaction is called **auto oxidation**.
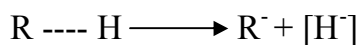
-Free redicals produced during initial reaction are highly reactive and further catalyze the reaction produced additional free radicals and causing a chain reaction.

Heavy metals such as copper, iron, cobalt to catalyze the oxidative degradation.
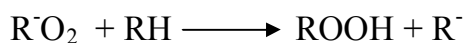
1.11 Steps Involved Oxidation Reaction:

1.11.1 Initiation:
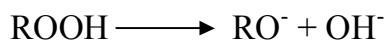
Formation of free redicals is taken place .

$$R ---- H \longrightarrow R^{\cdot} + [H^{\cdot}]$$

1.11.2 Propagation:

Here the free radical is regenerated and react with more oxygen.

$$R^{\cdot} + O_2 \longrightarrow R^{\cdot} \longrightarrow O_2$$

$$R^{\cdot}O_2 + RH \longrightarrow ROOH + R^{\cdot}$$

Hydroperoxide Decomposition:

$$ROOH \longrightarrow RO^{\cdot} + OH^{\cdot}$$

1.11.3 Termination:

 Free radicals react with each other resulting in inactive products.

$$R{-}O_2 + x \longrightarrow \text{Inactive product}$$

$$RO_2 + RO_2 \longrightarrow \text{Inactive product}$$
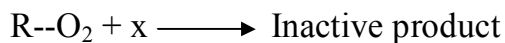
Example of Drugs Decomposed by Oxidation Path Ways: archis oil, Clove oil, ethyl olaeate.

1.12 Protection Against Oxidation:

Use of antioxidants:

Antioxidant are mainly of 3 types:

 1- The first group, probably inhibits the oxidation by reacting with free radicals.

Example:

Tocopheral, butylated hydroxyl anisole (BHA)

2- The second group, comprising the reducing agent have a lower redox potential than the drug or other substance that they should protect and are therefore more readily oxidized.

Example:

Ascorbic acid and iso ascorbic acid.

3- The third group, little antioxidant effect them self but enhance the action of true ontioxidant.

Example:

Citric acid – tartaric acid.

Use of Chelating Agent:

Example:

EDTA – Citric acid.

1.13 Photolysis:

Exposure to light cause substantial degradation of drug molecule.

When molecules are exposed to electromagnetic radiation they absorb light (photons) at characteristic ware length which cause increase in energy which can:

- Cause decomposition.
- Retained or transferred.
- Be converted to heat.
- Result in light emission at a new wave length (fluorescence. Phosphorescence).
- Natural sun light lies in wave length range (290 – 780 nm) of which only higher energy (UV) range (290 – 320) cause photo degradation of drugs.

Example of phototoxic drug:

Furosemide, acetazolemide, cynocobalamine

1.14 Protection:

- Use of amber colored bottles.

-Storing the product in dark, packaging in cartons also act as physical barrier to light.

- Coating of tablets with polymer films.

1.15 Stability Identifying Assays:

It is quantative analytical method which is based on the characteristic structural, chemical ,biological, properties of each active ingredient of drug product and that can differeniate between active pharmaceutical ingredient and its degradation product accurately.

1.15.1 Stabililty Indicating Assay Development:

Developing a stability indicating assay requires consideration of three aspects of the three method.

A- Obtaining a representative sample.
B- Choosing the separation techniques
C- Selecting the detectors.

1.15.2 OptainingArepresentative Sample:

Pure drug compound degrades into toxic compound.

Formaulation ………degradation $\longrightarrow$ drug (toxic) + inert (non toxic).

1.16 Preparation of sample:

- Forced degradation.
- Purposeful degradation.
- Drug is subjected to acid, base, heat, light, or oxidation.
- Goal is to degrade the drug.
- It should include 10 - 20% degradation and great or than 10 - 20% could result in secondary.

Degradants that will complicate the development process.

Dissolving portion of sample in 0.1 N hydrochloric acid for acid degradation and cllect sample at interval of 1, 2, 4, 8, 24 hrs.

- Similarly reaction is quenched in BASE.

- For oxidation(with peroxide), collect sample.

- Resulting sample is analyzed by measuring loss of parent drug.

- Auto sampler vials can also be used, injections at regular interval of 1hr.

- Observe sample change in time.

1.17 Separation:

Reverse Phase Chromatography is methoed of choice for stability indicating assays because the samples are generated in aqueous solutions.

*We should choose gradient elution for sample screening.

*Most commonly used solvent type are –acotonitrile, methanol.

Low and intermediate pH are generally obtained by use of phosphate buffer in the Ph 2.5- 6.5 range.

*If method involve mass spectroscopy(MS) detector at same point, select buffer that are MS compatible such as 0.1% trifluoroacetic acid .

1.17.1 Column temperature 35 -50 C:

Although each sample might contain only 4 or 6 significant degradants, different 30 degradation conditions can produce some of the same compounds in addition to unique degradation.

1.17.2 The Detectors:

UV detector remains the detector of choice for stability indicating assay.

MS detector can be very useful in identifying unknown peaks in the final method .

1.18 Potential Instability Issues OfFpp's:

- ✓ Loss\increase in concentration of API.
- ✓ Formation of (toxic) degradation products .
- ✓ Modification of any attribute of functional relevance.
- ✓ Alteration of dissolution time profile or bioavailability.
- ✓ Decline of microbiological status.
- ✓ Loss of package integrity.
- ✓ Reduction of lable quality.
- ✓ Loss of pharmaceutical elegance and patient acceptability.

1.18.1 Stability Testing and Product Development:

- Stability testing is an integral part of pharmaceutical development.
- It is evolutionary concept covering the life cycle of pharmaceutical product development.
- In early discovery phase the primary focus is to generate stability characteristics of a chemical \biological entity.
- In later stages, the goal is to establish shelf life for formulations packaged in final package intended for commercial introduction.

1.18.2 Stability Testing Principles Can be Subdivided in to Various Stagesof Drug Development:

- ❖ Discovery phase.
- ❖ Pre clinical stage.

- ❖ Pre-IND stage.
- ❖ IND stage.
- ❖ Product development stage.
- ❖ NAD stage.
- ❖ Approved product stage.
- ❖ Revised product stage.

1.18.2.1 Discovery Phase:

- To help select the most satisfactory chemical entity possessing the right pharmacological, toxicological and pharmaceutical profile.
- To select the right physical form (base, salt, ester).

1.18.2.2 Pre Clinical Stage:

- Pre liminary stability testing on all formulations must be carried out using stability indicating assays in accordance with GEPs.
- It requires an entrance assay prior to the initiation of toxiclogical testing and an exit assay, must be performed at the and of studies.

1.18.2.3 Pre –IND Stage:

Pre- formulation and stability evaluation of chemical entity is carried out according to ICH guidelines.

In addition to normal preformulation evaluations, forced degradition studies under highly stressed stability conditions is under takes .

1.18.2.4 IND Stage:

- Accelerated and normal storage temperature testing of drug substance and for clinical formulation must be initiated.
- The goal of these studies should be generate information to insure that the clinical formulations are likely to remain stable during the planned clinical studies.

1.18.2.5 Product Development Stage:

- Intermediate stability testing is done in this stage.
- Interim stability testing is conducted to establish the maximum time for which a drug product can be stored in interim containers for further processing.

1.18.2.6 NDA Stage:

- The stability of drugs should be evaluated in containers used for marketing.
- Care should be exercised in selection of the size, surface- to volume ratio of the container.

   Approved Product Stage:
- The goal of the stability program during this phase is to confirm or extending the expiration date.

1.18.2.7 Revised Product Stage :

Most products undergo post approval changes.

They may be internally driven or externally driven.

Internally driven: changing size and shape of dosage forms, changes in package design and others.

Externally driven: deletion of dyes, formulation changed and others.

1.19 Stability Protocol and Report:

1- Batch tested.

2- General information.

3- Container \ closure system.

4- Literature and supporting data.

5- Stability- indicating analytical methods.

6- Testing plan.

7- Test parameters.

8- Test results

9- Other requirements (post-approvalcommitments)

10- Conclusions.

1.20 Essential Definitions:

1.20.1 Re- test date:

The date after which samples of an API should be examined to ensure that the material is still in compliance with the specification and thus suitable for use in the Manufacture of given FPP.

1.20.2 Formal stability studies:

long term and accelerated (and intermediate) studies undertaken on primary and\or commitment batches according to a prescribed stability protocal to stablish or confirm the re- test period of an API or the shelf life of FPP.

1.20.3 Stress testing- forced degradation (API)

Studies undertaken to elucidate the intrinsic stability of the API. Such testing is part of the development strategy and is normally carried out under more server conditions than those used for accelerated testing.

1.20.4 Stress testing -forced degradation (FPP):

Studies undertaken to assess the effect of severe conditions on the FPP. Such studies include photostability and compatibility on APIs.

Photostability Testing of New Drug Substances and Products:

Photostability testing include:-

- Test on drug substance.

- Test on exposed drug product outside the immediate pack.

- Test on drug product in the immediate pack.

- Test on drug product in the marketing pack.

1.21 Container closure Systems:

- The containers should be tested in all directions.

- This is done for long term and accelerated stability testing.

- This is to ensure that there are no adverse effects from any interaction is produced.

1.22 Stability results:

* Astorage statement should be   proposed for the labeling.,(if applicable),which should be based on the stability evaluation of the API.

* Are- test period should be derived from the stability information.

* An API is considered as stable if it is within the defined \regulatory specifications.

1.23 Abbreviations:

API	Active Pharmaceutical Ingredient
DRA	Drug Regulatory Authority
EOI	Expression Of Interest
FDC	Fixed Dose Combination
FPP	Finished Pharmaceutical Product
GMP	Good Manufacturing Practices
ICH	International Conference on Harmonization
MA	Marketing Authorization
PQIF	Pharmaceutical Quality Information Form

1.24 Dosage Form Consideration:

| Dosage form	Evalution
Tablets	Appearance – colour – odour –assay – degradation – dissolution.
Hard gelatin capsules	Appearance- colour – odour – dissolution.
Soft gelatin capsules	Appearance- color- odour- degradation.
Emulsions	Appearance- color – odour- PH- viscosity- preservative content.
Oral solutions	Appearance color- odour- preservative content.
Oral suspensions	Appearance – colour – PH- preservative content- size and distribution of particle.
Oral powders	Appearance – colour- moisture.

In halations and nasal sprays	Appearance- calour- degradation product- uniformity –microscopic.
Topical- opthalamic-ointments-cerams- lotions- pastes-gels –solutions.	Appearance- colour -viscosity particle size – degradation –viscosity.

1.25 Salbutamol (Albuterol):

- Salbutamol is ashort acting B2 adrenergic recept against used for the relief of bronchospasm in condition such as asthma and chronicdostructine pulmonary disease it is marketed as ventolin among other brand names.

-The chemical name salbutamol sulphale is 1-[4-hydroxy-3- Hydroxy methyl phenyl]-2- (t- butylamino) – ethanol sulfate.

-Molecular formula is $(C_{13}H_{21})$-H_2SO_4 and molecular weight 576.7. After oral administration, approximately 50% of salbutamol is absorbed from the intestinal tract with aslower onset of action reaching apeak at about 2 hours after intake.
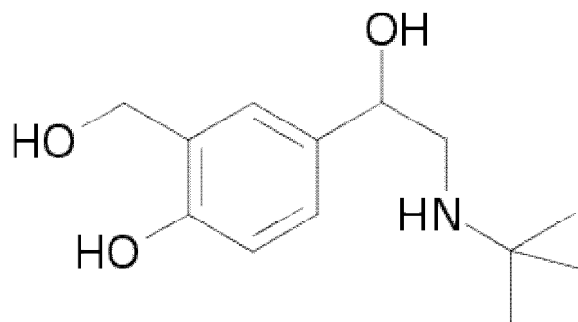

(RS) -4- {2- (tertbutyl amino)-1- hydroxy ethyl} -2- (hydroxyl methyl) phenol.
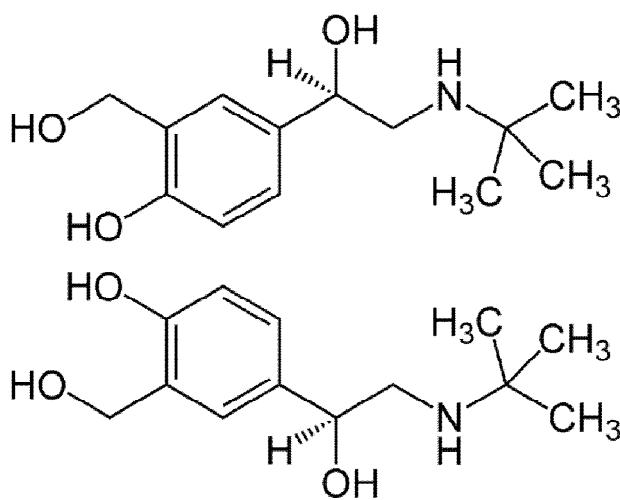
Formula C $_{13}H_{21}NO_3$ Mol mass 239.311.

1.25.1 Salbutamol Sulbhate:

Salbutamol sulphate is awhile or all most white odorless powder .It is soluble in 4 parts of water,slightly soluble in 95%alcohol in chloroform and solvent ether.Approximately 1.2mg of salbutamol sulphate is equivalent to 1mg of salbutamol.
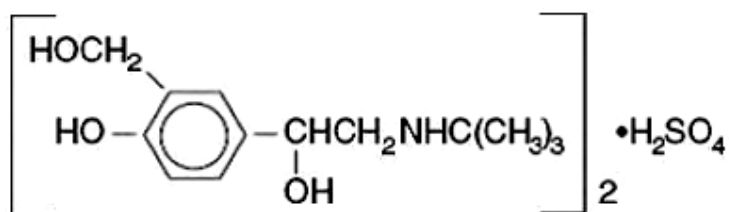
Molecular formula C$_{26}$H$_{44}$N$_2$O$_{10}$S.

1.25.2 Chemistry:

(R)-Salbutamol (top) and (S)-salbutamol

1.25.3 Structure-activity relationships:

The tertiary butyl group in salbutamol (or albuterol) makes it more selective for β$_2$-receptors. The drug is sold as a racemic mixture mainly because the (*S*)-enantiomer blocks metabolism pathways while the (*R*)-enantiomer shows activity.

1.25.4 Synthesis:

Salbutamol can be prepared from an acetophenone derivative which is itself derived from salicylic acid (hence the "sal" in salbutamol).
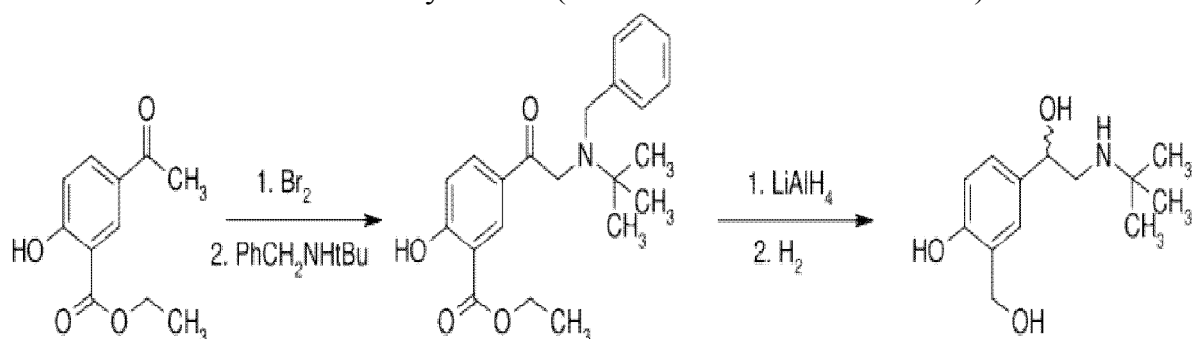
1.25.5 Uses for Salbutamol:

Albuterol is used to treat or prevent bronchospasm in patients with asthma, bronchitis, emphysema, and other lung diseases. This medicine is also used to prevent wheezing caused by exercise (exercise-induced bronchospasm).

Albuterol belongs to the family of medicines known as adrenergic bronchodilators. Adrenergic bronchodilators are medicines that are breathed in through the mouth to open up the bronchial tubes (air passages) in the lungs.

1.25.6 Before Using Salbutamol:

In deciding to use a medicine, the risks of taking the medicine must be weighed against the good it will do. This is a decision you and your doctor will make. For this medicine, the following should be considered.

1.25.7 Medical Uses:

Salbutamol is typically used to treat bronchospasm (due to any cause, allergen asthma or exercise-induced), as well as chronic obstructive pulmonary disease. Emergency medical practice commonly treats people presenting with asthma who report taking their salbutamol inhaler as prescribed with salbutamol. In general, people tolerate large dose well.

Other uses include in cystic fibrosis, along with ipratropium bromide, acetylcysteine, and pulmozyme and subtypes of congenital myasthenic syndromes associated to mutations in *Dok-7*.

As a β_2-agonist, salbutamol also finds use in obstetrics. Intravenous salbutamol can be used as a tocolytic to relax the uterinesmooth muscle to delay premature labor. While preferred over agents such as atosiban and ritodrine, its role has largely been replaced by the calcium-channel blocker nifedipine, which is more effective, better tolerated and orally administered.

Salbutamol is used to treat acute hyperkalemia as it stimulates potassium to flow in cells thus lowering the level in the blood.

Salbutamol has also been trialled in spinal muscular atrophy where it appears to show modest benefits. The drug is speculated to modulate the

alternative splicing of the *SMN2* gene, increasing the amount of the SMN protein whose deficiency is regarded as the root cause of the disease.

1.25.8 Adverse effect:

The most common side effects are fine tremor, anxiety, headache, muscle cramps, dry mouth, and palpitation. Other symptoms may include tachycardia, arrhythmia, flushing, myocardial ischemia (rare), and disturbances of sleep and behaviour. Rarely occurring, but of importance, are allergic reactions of paradoxicalbronchospasm, urticaria, angioedema, hypotension, and collapse

1.25.9 Dosing:

The dose of this medicine will be different for different patients. Follow your doctor's orders or the directions on the label. The following information includes only the average doses of this medicine. If your dose is different, do not change it unless your doctor tells you to do so.

The amount of medicine that you take depends on the strength of the medicine. Also, the number of doses you take each day, the time allowed between doses, and the length of time you take the medicine depend on the medical problem for which you are using the medicine.

- **1.25.9.1 For inhalation aerosol dosage form (inhaler):**
  - **1.25.9.1.1 For preventing bronchospasm:**
    - Adults, teenagers, and children 4 years of age and older—Two puffs every 4 to 6 hours as needed.
    - Children younger than 4 years of age—Use and dose must be determined by your child's doctor.
  - **1.25.9.1.2 For preventing exercise-induced bronchospasm:**

- Adults, teenagers, and children 4 years of age and older—Two puffs taken 15 to 30 minutes before exercise.
- Children younger than 4 years of age—Use and dose must be determined by your child's doctor.
- For inhalation solution dosage form (used with a nebulizer):
  - **1.25.9.1.3 For preventing bronchospasm:**
    - Adults and children older than 12 years of age—2.5 milligrams (mg) in the nebulizer 3 or 4 times per day as needed.
    - Children 2 to 12 years of age—0.63 to 1.25 mg in the nebulizer 3 or 4 times per day as needed.
    - Children younger than 2 years of age—Use and dose must be determined by your child's doctor.

1.25.10 Storage:

Store the canister at room temperature, away from heat and direct light. Do not freeze. Do not keep this medicine inside a car where it could be exposed to extreme heat or cold. Do not poke holes in the canister or throw it into a fire, even if the canister is empty.

Keep the medicine in the foil pouch until you are ready to use it. Store at room temperature, away from heat and direct light. Do not freeze.

Store the medicine in a closed container at room temperature, away from heat, moisture, and direct light. Keep from freezing.

Keep out of the reach of children.

1.25.11 Salbutamol Side Effects:

Along with its needed effects, a medicine may cause some unwanted effects. Although not all of these side effects may occur, if they do occur they may need medical attention.

Check with your doctor immediately if any of the following side effects occur:

1.25.11.1 More common

- Fast, irregular, pounding, or racing heartbeat or pulse
- shakiness in the legs, arms, hands, or feet
- trembling or shaking of the hands or feet

1.25.11.2.Less common

- Abdominal or stomach pain
- bladder pain
- bloody or cloudy urine
- chest discomfort
- chest pain
- cough or hoarseness
- cough producing mucus
- diarrhea
- difficult or labored breathing
- difficulty with swallowing
- heezing

1.25.11.3 Rare

- Hives or welts
- large, hive-like swelling on the face, eyelids, lips, tongue, throat, hands, legs, feet, or sex organs
- noisy breathing
- redness of the skin
- swelling of the mouth or throat
- trouble breathing

1.25.12 Mechanism of action:

► Salbutamol stimulates β_2 adrenergic receptors which are predominant receptors in bronchial smooth muscle of the lung. Stimulation of β_2 receptors leads to the activation of enzyme adenylcyclase that form cyclic AMP (adenosine-mono-phosphate) from ATP (adenosine-tri-phosphate). This high level of cyclic AMP relaxes bronchial smooth muscle and decreases airway resistance by lowering intracellular ionic calcium concentrations. Salbutamol relaxes the smooth muscles of airways, from trachea to terminal bronchioles.

► High level of cyclic AMP are also inhibits the release of bronchoconstrictor mediators such as histamine, leukotreine from the mast cells in the airway.

- See more at: http://salbutamol.org/#sthash.GI1XOJjW.dpuf

1.25.13 Overdose:

The most common symptoms of overdose with salbutamol are tremor, palpitation and tachycardia. It may also produces arrhythmias,

hypertension, angina, seizures, nervousness, fatigue, malaise, headache, dizziness, sleeplessness, dry mouth and even cardiac arrest.

Treatment is symptomatic with discontinuation of salbutamol is needed. A cardio-selective beta receptor blocking drug (atenolol, metoprolol) may be given by intravenous injection in patients presenting with tachycardia and palpitation. In general, beta receptor blocking drugs should be used cautiously as they may cause bronchospasm in sensitive persons. Hypokalaemia (decrease potassium level in the blood) may occur following overdose with salbutamol. Serum potassium level should be monitored.

Aim and Objectives:

1- To separate components of Ventolin® tablets by Automatic HPLC.

2- To indentify Salbutamol sulphate by IR spectrometer and UV spectroscopy at different PH values range from 1-14.

Chapter Two

Instruments and methods

Instruments And methods

2-1Instruments

2-1-1Infra Red Spectrometer

Name of the instrument: FT-IR Spectrometer

- Model No: FI-IR-4100

- Make: JASCO

- Date of issue: January 2011

- IOP No: 1-49

- Supersedes-New

2-1-1-1 Different components:-

- The main IR instrument (4100)

- The Soft ware (spectra manger)

2-1-2 Automatic Highperformanceliquid chromatography

Name of Instrument   :   Automatic   High   performance liquid Chromatography

Make: Japan

2-1-2-1 Basic Components

- **Pump**

- SYTAM

- S/121 solvent Delivery system

- S 5200 sample injector

- **Column**

- PRp-1

- Part No = 79427

- Serial No = 15904

- Hamicton

- 17.2. 2014 – BP Methods

Detector:

- Peak simple chromatography Date system uv/vis Detector 53200

2-1-3Ultra Violet Spectrometer

Name of instrument: Uv/visible spectrometer

Make: SHIMADZU

Mode No: UV- 1800

Date of Issue: June /2009

Inst, No/ I OP No. 1-45

2-1-3-1 Basic component:-

- The UV- 1800 instrument

- The Computer

- Printer (HP DeskJet 845C)

2-1-4Balance

- Sartorius AG

- Make Germany

- CPA 124s

- Weight Capacity :Max 120g

- Density : 0.1 mg

- Serial Number : 26605548

2-2 Methoeds of preparation

2-2-1 Working standard:

- Date of preparation:-   24-11-2011

- Working STD NO = WS/ Ami/BP. CAT302

- Item: salbutamol sulphate

- Valid up to 24.11.2014

2-2-2 Method of Infra Red Spectroscopy

Aminum amount of working standard was taken then milled with potassium bromide and road by IRspectrometer.

The spectrum was obtained compered with Refrecespectrum of salbutamol sulphate

2-2-3 Methods ofHigh performance liquid chromatography

2-2-3-1 Preparationof Standard Solution:

0.048g of working standard was weighted dissolved completed to 100ml vlometric flask by mobile phase to mark.

2-2-3-2 Preparation of sample:

MeanweightX' = 0.13013 g

0.13013 X 10 tablets = 1.3g

10 tablets of sample were crushed then 1.3g of sample was weighted and dissolved by Mobile phase. Put in shaker about 90 minutes after completed in 100ml volumetric flask by mobile phase to mark. the sample was filtred by double filter paper. Finally the filtrate was read byAuto HPIC.

2-2-3-3 Preparation of Mobile Phase:-

- Ammonium acetate 0.05 M in 300 ml distill water.
- Filter distilled water 650 ml
- 2- Propanol 50 ml
- The PH was adjustedat PH = 4.5 byaddingglac. HAC
- Wg of ammonium acetate = $\dfrac{M.V \text{ X } MWt}{1000}$

$$= \dfrac{0.05 \text{ X } 300 \text{ X } 77.08}{1000} = 1.16g$$

2-2-3-4 Methods of Preparation

HPIC:	Methods of Preparation
Column Type	Ass column (20CmX5mm) packed with spertical particles of silica, 5 micrometer in diameter the surface of wish has been modified with chemically bonded nitrile groups(HamiltonPRp-1is suitable).
Mobile Phase	Amixture of 65 volumes of water 30 volumes of 0.05 M ammonium acetate and 5 volume of propanl-2.ol adjust to PH 4.5 with glacial acetic acid
Flow rate	1.0 ml/ Minute
Detection wave length	276 nm
Injection volume	20.0 micro litter
Preparation of solution (1)	Contained 0.048% w/v of 2-tert.buty (amino-1-[4-hydroxy-3-methylphenyl]ethanol [Sulphate BPCRS and 0.048% w/v of salbutamol sulphate BRCRS in Methanol (10%).
Solution (2)	Contained 0.048% W/V of Salbutamol sulphate BPCRS in water (0.048g) of salbutamol sulphate BPCRS was weighted dissolved and diluted to 100 ml with mobile phase
Solution (3)	10 tables were Shaken with 100ml of M.ph for 90 minute added sufficient water to produce asolution containing the equivalent of 0.0040 W/V of salbutamol mixed and filtered - The test is not valid if the resolution factor between the two principle peaks in chromatogram obtained with solution (1) is less than 1-5. - 3 standard were Injected and calculated the mean for assay calculation injected solution (1) third and calculate the assay individually.

2 -2-4 Method of Ultra Violet Spectroscopy

2-2-4-1 Preparation of sample:

0.1g of working standard was weighted in small cap dissolved with distilled water then completed in 100ml volumetric flask to 35 mark 5ml of solution was taken and completed to 50 ml volumetric flask by buffer to mark and was read by UV spectrometer

2-2-4-2 Preparation of blank:

75 ml of potassium di hydrogen phosphate was added to Sodium hydroxide orortho phosphoric acid

2-2-4-3 Preparation of Buffer:

2-2-4-3-1 Phosphate buffer

- Potassium di hydrogen phosphate concentration 0.2 M

- Wg = $\underline{M \times V \times Mwt}$

$$ 1000$$

= $\underline{0.2 \times 1000 \times 136.09}$

$$ 1000 \qquad = 27.218 \ g$$

27.818g of potassium di hydrogen phosphate was weighted then dissolved in 1000ml volumetric flask completed by distill water to marks

2-2-4-3-2 Preparation of Sodiumhydroxide

Wg= $\underline{M \times V \times Mwt}$

$$ 1000$$

$$= \underline{0.2 \times 250 \times 40}$$

$$1000 \qquad = 2g$$

2g of Sodium hydroxide was weighted in small cap disso lved   with distilled water and the volume was completed in 250ml volumetric flask to mark.

2-2-4-4 Adjustment of logarithm hydrogen

PH	Phosphate buffer	Sodium hydroxide
1)	75 ml of phosphate buffer was adjusted by ortho phosphoric acid. 5ml of sample was taken and completed with buffer in50mlvolumetric flask to mark	
2)	75 ml of phosphate buffer was adjusted by ortho phosphoric acid. 5ml of sample was taken and completed with buffer in50mlvolumetric flask to mark	
3)	75 ml of phosphate buffer was adjusted by ortho phosphoric acid. 5ml of sample was taken and completed with buffer in50mlvolumetric flask to mark	
4)	75 ml of phosphate buffer was adjusted by ortho phosphoric acid. 5ml of sample was taken and completed with buffer in50mlvolumetric flask to mark	

5)	75 ml of phosphate buffer was adjusted by ortho phosphoric acid. 5ml of sample was taken and completed with buffer in50mlvolumetric falsk to mark	
6)	75 ml of phosphate buffer was adjusted by ortho phosphoric acid. 5ml of sample was taken and completed with buffer in50mlvolumetric flask to mark	
7)		75 ml of phosphate buffer was adjusted by Sodium hydroxide 5ml of sample was taken and completed with buffer in50mlvolumetric flask to mark
8)		75 ml of phosphate buffer was adjusted by Sodium hydroxide 5ml of sample was taken and completed with buffer in50mlvolumetric flask to mark
9)		75 ml of phosphate buffer was adjusted by Sodium hydroxide 5ml of sample was taken and completed with buffer in50mlvolumetric flask to mark
10)		75 ml of phosphate buffer was adjusted by Sodium hydroxide 5ml of sample was taken and completed with buffer in50mlvolumetric flask to mark

11)		75 ml of phosphate buffer was adjusted by Sodium hydroxide 5ml of sample was taken and completed with buffer in50ml volumetric flask to mark
12)		75 ml of phosphate buffer was adjusted by Sodium hydroxide 5ml of sample was taken and completed with buffer in50mlvolumetric flask to mark
13)		75 ml of phosphate buffer was adjusted by Sodium hydroxide 5ml of sample was taken and completed with buffer in50mlvolumetric flask to mark
14)		75 ml of phosphate buffer was adjusted by Sodium hydroxide 5ml of sample was taken and completed with buffer in50mlvolumetric flask to mark

Chapter Three

Instruments and methods

3-1 Infra Red Spectroscopy:

The spectrum of sample of salbutamol was identical the spectrum of standard of salbutamol in finger print region.

- IR (KBr) $\bar{V}=$

- N – H for secondary amine group at 3330 cm^{-1} .
- O-H at 3500 cm^{-1}.
- C-H stretching at 2999 cm^{-1}.
- C--- C at 1610 cm^{-1}.
- C-O at 1120 cm^{-1}.
- C-N at 1210 cm^{-1}.

3-2 AutomaticHigh Performance Liquid Chromatography:

From the peaks the retension time of standard was similar to retension time of sample "4.050" min this was qualitative analysis.

Quantitative analysis.

$$X^- \text{of STD} = \frac{1802.5380 + 1806.445 + 1802.9860 + 1778.1610 + 1780.4450 + 1806.09010}{6}$$

$X^-$of STD = 1796.377583

$$X^- \text{of sample} = \frac{1890.6915 + 1893.6870 + 1906.7160}{3}$$

$$= 1897.0315$$

$$\text{Assay} = \frac{\text{Area sample}}{\text{Area STD}} \quad \text{X purity}$$

$$\underline{\frac{1897.0315}{1796.377583}} \quad \text{X99.08} \quad = 104.6\%$$

Scale 95% - 105%

From the peaks it was found that the area under the peak of standard was similar to area under the leak of sample.

3-3 Ultra Violet Spectroscopy:

Sample analysis was done at 276 nm when was used shimadzu (uvspectro photometer) scanning the pH 1.2.3.4.5.6.7.8. It was found that the real λmax respectively equal 276-40, 275.80, 276.40, 276.60, 276.60, 276.50,276.80,276.80it was found that the values of λmax were changed in short range. This values in acidic medium.

When was scanned in strong basic medium at pH 9.10.11.12.13.14 it was found that real λmax respectively equal 279.40, 285.40, 294.20, 294.60, 295.80, 295.20 that means the values of λmax were increased in basic medium.

Investigation were carried out to ascertain the relative importance of the described mechanism in iontophoretic transport using an ionizable drug salbutamol sulphate which has two real λmax were appeared at pH 9(for amino group) 10 (for phenolic group)

Ionization of salbutamol salphate varies with pH hence the rate and extent of transport across the skin can be enhanced, controlled and manipulated by the application of factors like anodal and cathodal current at varied pH of donor solution and current densities.At pH 1.2.3.4.5.6.7.8.9.Divation Bearlambert law was appeared at λmax respectively equal 226, 224.80, 225.20, 224 ,224.80, 232 and at pH 10, 11, 12, 13, 14, divation bear

lambert law was appeared at λmax respectively equal 244.20 (242.60, 213.40), 241.20, (247.80, 218.20) 239.40.

That is due tocathodalionphoretic flux was significantly higher than corresponding anodal flux.

At pH 7, 8 divation bear lambert law were dispeared due to anodaliontophoretic flux was not signifycantly different from passive fusion

iontophoresis (anodal and cathodal) enhances the transport salbutamol sulphate through hairless mice skin as compared to passive diffusion.

Anodaliontophoresis at pH 7 was more effective in transported of salbutamol sulphate across skin as compared to cathodaliontophoresis at pH11.

The decomposition of salbutamol sulphate was less in phosphate Buffer and high in sodium hydroxide due to the amino and phenolic groups.

Chapter Four

Conclusion & Reference

Conclusion:

Stability studies should be planned on the basis of pharmaceutical R and D and regulatory regulatory requirements.

* Forced degradation studies reveal the intrinsic chemical properties of the API, while formal stability studies establish the retest date.

* The shelf life (expiry date) of FPPs is derived from formal stability studies.

* Variability and time tr

ends of stability data must be evaluated by the manufacture in order to propose a retest date or expiry date.

Reference:

1- K-wolters-Rehmington the science and practice of pharmacy "edition volume 2005 puplished in Philadelphia page no-1025-1033.

2- Sachinkumar January 2011.

3- Who model list of essential medicines .World Health Organization October 2013 retrived 22 April 2011.

4- J.s ptrick:Martin's physical pharmaceutical sciences page no 428-432.

5- K-herbert A;LLachman, the Theory and practice of Industrial pharmacy.

6- Mahoney,B.A:Smith,W.A;LO,D.Tsoi,Tonelli,M.;Clase,C.2005.

7- Collin,DavidT.;Hartley,David.;Jack,David.;Lunts,LawrenceH.C.;Press1970Jurnal of Medicinal Chemistry 13(4).

8- Mehta,Akul.Medicinal Chemistry of the Peripheral Nervous System 20/10/2010.

9- Ahee,P.2000".The management of hyperkalaemia in the emergency department"Journal17(3) 188-191.

10- CarterWJ,LynchME(September1994.

11- Rossi,S(2004).Australion Medicines Handbook.

12- Caruso,J.(20January 2005).

13- "Albuterol aids resistance exercise in reducing un loading – induced anhle extensor strength losses"Journal of applied physiology 98(5):1705-1711.

14- Ventolin solution for IV infusion retrieved 18/9/2013.

15- Albuterol Sulphate .The internet drug Index 6/12/2008 retrieved 13/7/2014

16- Salbutamol ergogenic effects of saibutamol.Retrieved 20/10/2010