



STABILITY THEORITICAL CONSIDERATION DEGRADATIVE PATHWAYS, STABILITY INDICATING ASSAYS

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DRUG STABILITY



INTRODUCTION

DEFINATION

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

OR

The capacity or the capability of a particular formulation in a specific container to remain with in particular **chemical** , **microbiological** , **therapeutically** , and **toxicological** specifications.

USP defines stability of pharmaceutical product as , “extent to which a product retains with in specified limits and throughout its period of storage and use (i.e. shelf life).

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} :

$$t_{90} = \frac{(a-0.9a)}{k_o} = \frac{0.1 a}{k_o}$$

Where ,

a = initial concentration .

k_o = specific rate constant for zero order reaction.

The units of shelf life is time /conc.

FACTORS AFFECTING DRUG STABILITY

THE PRIMARY FACTORS AFFECTING STABILITY :

1.TEMPERATURE: High Temperature accelerate oxidation, reduction and hydrolysis reaction which lead to drug degradation

2. pH:

- Many drugs are stable between pH 4 and 8.
- Weakly acidic and basic drugs show good solubility, they also decompose faster when they are ionized.

So, if the pH of a drug solution has to be adjusted to improve solubility and the resultant pH leads to instability then a way out of this tricky problem is to introduce a water miscible solvent into the product.

It will increase stability by:

- suppressing ionization
- reducing the extreme pH required to achieve solubility
- enhancing solubility and
- reducing the water activity by reducing the polarity of the solvent.

- For example: 20% propylene glycol is placed in chlorthalidone injection for this purpose.

3. MOISTURE:

- a. Water catalyses chemical reactions as oxidation, hydrolysis and reduction reaction.
- b. Water promotes microbial growth.

4. **LIGHT:** Affects drug stability through its energy or thermal effect which lead to **oxidation**.

5. **PHARMACEUTICAL DOSAGE FORMS:** Solid Dosage forms are more stable than liquid dosage forms for presence of water.

6. **DRUG INCOMPATIBILITY:** Reactions between components of pharmaceutical dosage forms it self or between these components and cover of the container .

7. **OXYGEN:** Exposure of drug formulations to oxygen affects their stability

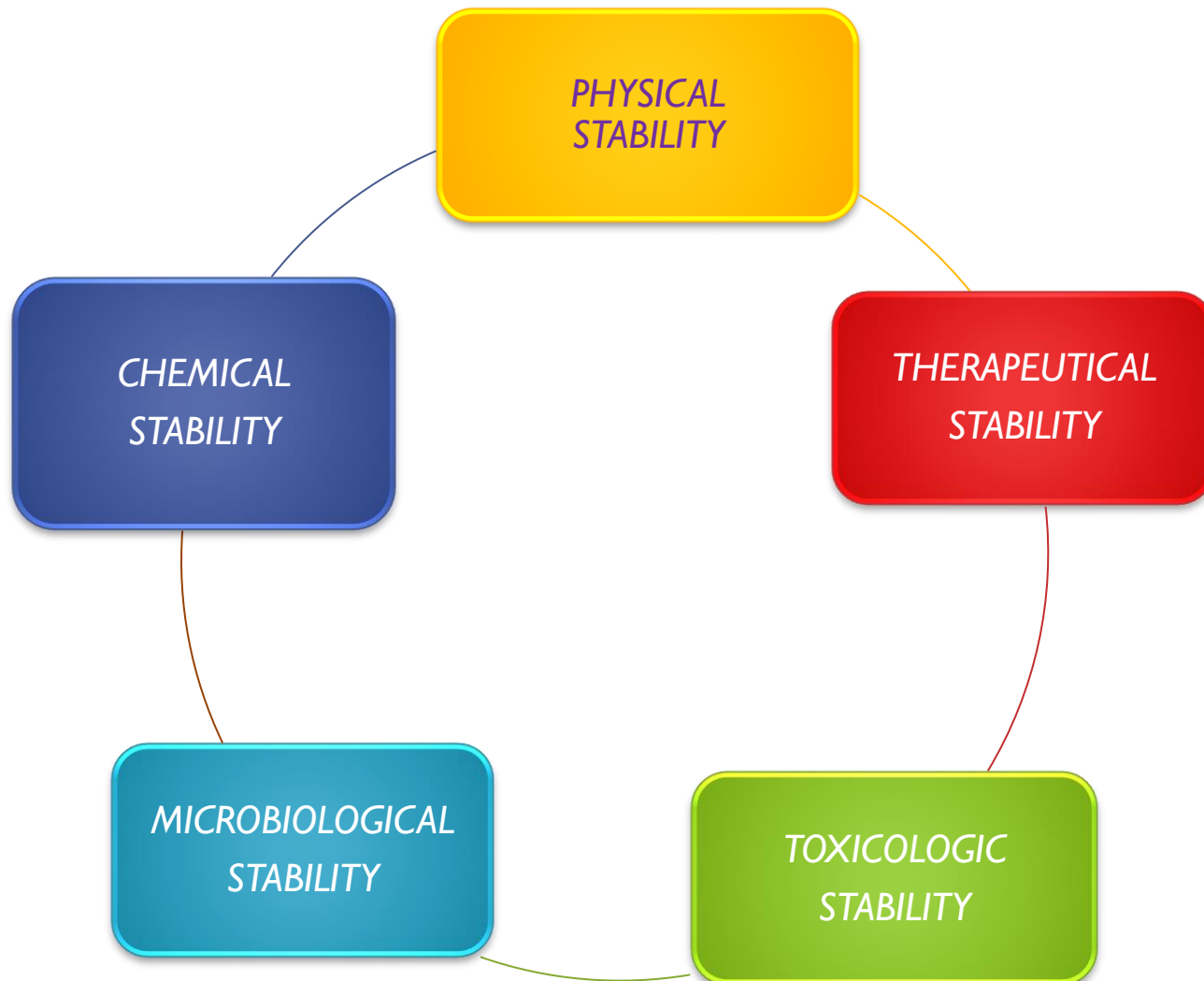
The Major Factors Affecting Drug Stability Are :

- ❖ Particle size (suspension and emulsion)
- ❖ Additives and excipients
- ❖ Molecular binding
- ❖ Diffusion of drugs

OBJECTIVES

- ❖ To determine and maintain maximum expiration date/ shelf life.
- ❖ To provide better storage condition.
- ❖ To determine the packaging components.
- ❖ Together information during preformulation stage to produce a stable product.

TYPES OF STABILITY



TYPES OF STABILITY THAT MUST BE CONSIDERED FOR ANY DRUG

❑ PHYSICAL STABILITY

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

❑ CHEMICAL STABILITY

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

❑ MICROBIOLOGICAL STABILITY

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

❑ THERAPEUTIC STABILITY

Therapeutic activity remains unchanged .

❑ TOXICOLOGIC STABILITY

No significant increase in toxicity occurs.

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.

ICH GUIDELINES FOR STABILITY TESTING

The ICH has released six guidelines for stability studies as indicated in table :

ICH GUIDELINES	TITLE
Q 1 A	Stability testing of new drug substances and products (second revision)
Q1B	Stability testing : photo stability testing of new drug substance and products.
Q1C	Stability testing for new dosage forms
Q1D	Bracketing and matrixing designs for stability testing of drug substances and products
Q1E	Evaluation of stability data
Q1F	Stability data package for registration application in climatic zones III and IV

CLIMATIC ZONES

AS per ICH and WHO guidelines world has been divided into four zones :

ZONE 1 - TEMPERATE

ZONE 2 - SUBTROPICAL WITH POSSIBLE HIGH HUMIDITY

ZONE 3 - HOT, DRY

ZONE 4 - HOT, HUMID

STABILITY STORAGE CONDITION :

According to WHO, long term stability testing during and beyond expected shelf life under storage conditions in the intended market.


LONG -TERM STABILITY STUDIES:

STORAGE CONDITIONS		
TEMPERATURE (°C)	RELATIVE HUMIDITY%	MINIMUM TIME
25°C ± 2°C	60 ± 5%	12 MONTHS
30°C +/- 2°C	30 +/- 5%	6 MONTHS

ACCELERATED STABILITY STUDIES:

STORAGE CONDITIONS		
TEMPERATURE (°C)	RELATIVE HUMIDITY%	MINIMUM TIME
40°C +/- 2°C	75 +/- 5%	6 MONTHS

Substances and drugs products intended to be stored in a refrigerator .



STABILITY STUDIES FOR PHARMACEUTICAL FORMULATION

TABLET

- ❖ Stable tablets retain their original size, shape, weight, roughness, colour-variation, cracking under normal handling and storage conditions throughout their shelf life.

FRIABILITY TEST : by ROCHE FRIABILATOR

Maximum weight loss should not be more than 1%.

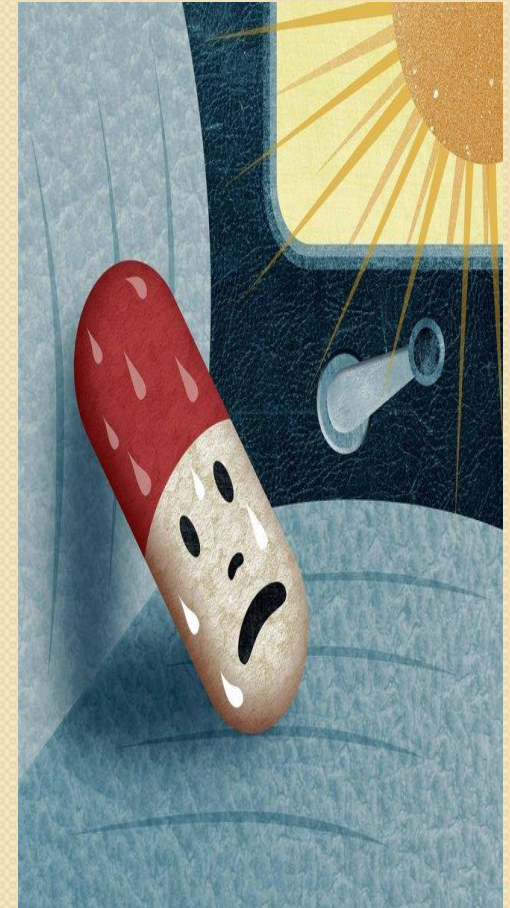
HARDNESS TEST : by apparatus are: the Monsanto tester, the Erweka tester, the Strong-Cobb tester, Pfizer tester and Schleuniger tester shows resistance to crushing strength.

COLOR STABILITY : by Colorimeter, Reflectometer with heat, Sunlight and Intense Artificial Light.

- ❖ Uniformity of weight, odour, Texture and moisture content, humidity effects are also Studied during a tablet test.

GELATINE CAPSULE

- Gelatin capsules are found to be stable in dry conditions but they rapidly reach equilibrium with the atmospheric conditions under they are stored.
- This shows gelatin capsules are largely effected by **TEMPERATURE AND HUMIDITY** and susceptibility to microbial degradation .
 - **Humidity** -capsule shell softens and becomes sticky.
 - **Dried**- capsule shell becomes brittle and crack.
- Soft Gelatin Capsule have Relative Humidity 20- 30% at 21 to 24°C.
- Hard Gelatin Capsule contain 13 to 16% moisture.
- Hard gelatin capsule are tested for **Brittleness, Dissolution, water content** and **level of microbial contamination**.





➤EMULSIONS

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.

For suspensions, redispersibility, rheological properties, mean size and distribution of particles should be considered .

➤NASAL SPRAYS : solution and suspensions

Clarity (for solution), level of microbial contamination, pH, Particulate matter, unit spray medication, content uniformity, droplet and particle size distribution, weightloss, pump delivery.

Microscopic evaluation (for suspension), foreign particulate matter and extractable/ leachable from components of the container , closure and pump.

➤ TOPICAL, OPHTHALMIC & OTIC PREPARATION:

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

Preparations should be evaluated for clarity,
Homogeneity, pH, resuspendibility for lotions
Consistency, viscosity, particlesize distribution,
level of microbial contamination / sterility and weight loss.

Should include the following additional attributes for otic and ophthalmic preparation :

Sterility, particulate matter, and extractable.

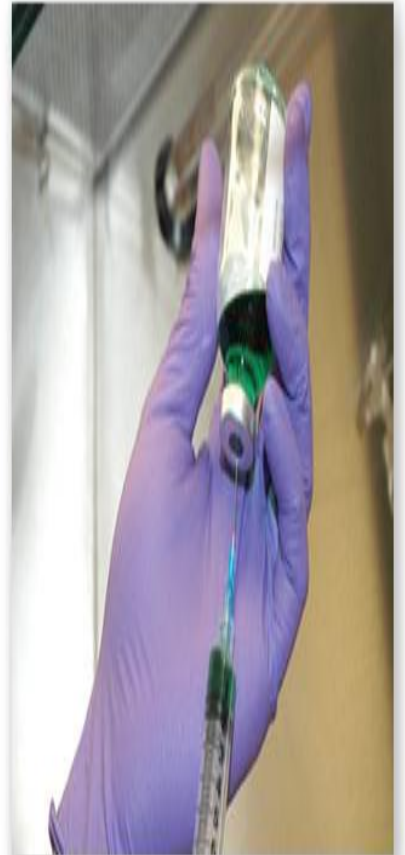


SUPPOSITORIES

- **Softening range , dissolution (at 37°C).**

PARENTERALS

- **Color , clarity (for solutions), particulate matter , pH, sterility ,pyrogen (endotoxins) .**
- **Stability studies for powders for injection solution include color monitoring reconstitution time and water content to be performed at regular intervals .**



DEGRADATION MAY BE OF TWO TYPES

- PHYSICAL DEGRADATION
- CHEMICAL DEGRADATION
 - OXIDATION
 - DECARBOXYLATION
 - PHOTOLYSIS
 - RACEMIZATION
 - HYDROLYSIS

➤ PHYSICAL DEGRADATION

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

Example :

Nitroglycerine Tablets

- ❑ **LOSS OF WATER :** Water loss from liquid preparation (o/w emulsion) leads to changes in stability .
- ❑ It causes crystallization of drug product which may lead to increase in potency and decrease in weight.

Example :

water evaporates from $\text{Na}_2\text{SO}_4 \cdot \text{BORAX}$.

- ❑ **WATER ABSORBANCE:** Pharmaceutical formulations which are hygroscopic in nature absorb the water from its external environment leads to degradation .

Example:

Gelatin capsule, deliquescent salts like – CaCl_2 , Potassium citrate.

- ❑ **POLYMORPHISM:** A stable crystal form is effected (it may loosen) leads to the formation of polymorph and cause instability in formulation.

This may lead to alteration in solubility, dissolution of drug .

- ❑ **COLOR CHANGE:** Loss or development of color may occur (due to change in pH, use of reducing agent, exposure to light).

■CHEMICAL DEGRADATION

➤Chemical degradation of a dosage form occurs through several pathways like –hydrolysis, oxidation, decarboxylation, photolysis, racemization which may lead to lowering of therapeutic agent in the dosage form formation of toxic product and decreased bioavailability etc.

HYDROLYSIS

- Most important in systems containing water such as emulsion , suspension , solutions , etc.
- Also for drugs which are affected by moisture (water vapour) from atmosphere.
- It is usually catalysed by hydrogen ion(acid) or hydroxyl ion(base).
- In this active drug is decomposed with solvent.
- Usually solvent is water some time reaction may involve pharmaceutical co-solvents such as ethyl alcohol or poly-ethylene glycol.
- Main classes of drugs that undergo hydrolysis are the ESTERS, AMIDE, ALKALI, ACID.

➤ESTER HYDROLYSIS : involve acyl – acid cleavage.

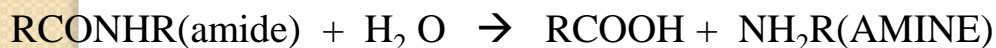
➤ Example of drugs: aspirin ,atropine , physostigmine , procaine..

➤ $R \cdot COOR \text{ (ester)} + H_2O \rightarrow RCOOH \text{ (acid)} + HOR \text{ (alcohol)}$

➤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 .



PROTECTION AGAINST HYDROLYSIS

- Avoiding **contact with moisture** at time of manufacture.
- **Packaging in suitable** moisture resistant packs such as strip packs and storage in controlled humidity and temperature.
- 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 drugs such as penicillin and derivatives can be prevented by formulating them in the dry powder form for reconstitution or dispersible tablets instead of a liquid dosage form such as solutions or suspensions.

OXIDATION

- Oxidation is the loss of electrons while reduction is the gain of Electrons.
- Either the addition of oxygen or removal of Hydrogen .
- Oxidation is controlled by environment i.e; light, trace elements, oxygen and oxidizing agent occurs when exposed to atmospheric oxygen.

EXAMPLE OF DRUGS DECOMPOSED BY OXIDATION PATHWAYS

- Archis oil , clove oil , ethyl oleate , Heparin , Ascorbic acid, Morphine, Vitamin A , Vitamin B₁₂ , etc.

AUTOXIDATION

- The reaction between the compounds and molecular oxygen is required for initiating the chain reaction is called **autoxidation** .
- Free radicals 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 , and nickel have been known to catalyze the oxidative degradation .
- Heat and light further influence the kinetics of oxidative degradation processes.

PROTECTION AGAINST OXIDATION

USE OF ANTIOXIDANTS :

Antioxidants are mainly of 3 types :

1. The first group probably inhibits the oxidation by **reacting with free radicals**.
Example –tocopheral ,butylated hydroxyl anisole (BHA) , butylated hydroxyl toluene's (BHT){ Concentration : 0.001 – 0.1% }.
2. The second group comprising **the reducing agents** , 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 , Potassium Or Sodium Salts of Metabisulfite.
3. The third group, little **antioxidant** effect themselves but enhance the action of true antioxidant .
Example -- Citric acid , tartaric acid , disodium edetate and lecithin .

USE OF CHELATING AGENT when heavy metals catalyze oxidation .

Example -- EDTA , citric acid , tartaric acid form complexes.

PHOTOLYSIS

Exposure to light cause substantial degradation of drug molecule.

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

- Cause decomposition.

- Retained or transferred.

- Be converted to heat .

- Result in light emission at a new wavelength (fluorescence phosphorescence).

- Natural sun light lies in wavelength range (290-780nm) of which only higher energy (UV) range (290-320nm) cause photo degradation of drugs.

❑ Example of phototoxic drugs:

Furosemide , acetazolamide , cynocobalamine .

❖ EXAMPLE

- a) SODIUM NITROPRUSIDE in aqueous solution (which is administered by IV -infusion for management of acute hypertension).
- b) If protected from light it is stable to at least 1yr.
- c) If exposed to normal room light it has a shelf life of 4 hrs.

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.

DEGRADATIVE PATHWAYS OF PHARMACEUTICAL DOSAGE FORMS

- ❑ Degradation of active drug leads to lowering of quantity of the therapeutic agent in the dosage form.
- ❑ It may not be extensive , a toxic product formation may take place due to decomposition instability of drug product can lead to a decrease in its bioavailability .
- ❑ Changes in physical appearance of given dosage form may take place.
- ❑ Degradation may increase or may decrease the potency of drug.

PACKAGING AND PRESERVATIVES

Packaging of the drug product is very important when its stability is being considered.

The immediate container and closure are particularly important in affecting product stability.

Glass, plastic, rubber (natural and synthetic) and metal are the four types of containers commonly utilized for packing drug products.

1.GLASS

Glass is resistant to chemical and physical change and is the most commonly used material, but it has the limitations of :

1. Its alkaline surface may raise the pH of the product.
2. Ionic radicals present in the drug may precipitate insoluble crystals from the glass
3. The clarity of the glass permits the transmission of high energy wavelength of light which may accelerate decomposition.

All these limitations are overcome by the technologists in the following way:

1. the first problem is overcome by the use of Borosilicate glass which contains fewer reactive alkali ions.
2. Treatment of glass with chemicals or the use of buffers helps in overcoming the second problem
3. Amber coloured glass which transmits light only at wavelengths above 470 nm is used for photolytic drug products.

2. PLASTICS

Plastics include a wide range of polymers of varying density and molecular weight, each possessing different physicochemical characteristics.

The problems with plastic are:

1. Migration of the drug through the plastic into the environment.
2. Transfer of environmental moisture, oxygen, and other elements into the pharmaceutical product.
3. Leaching of container ingredients into the drug.
4. Adsorption or absorption of the active drug or excipients by the plastic.

3. METALS

Various alloys and aluminium tubes may be utilized as containers for emulsions, ointments, creams and pastes.

- They may cause corrosion and precipitation in the drug product.
- Coating the tubes with polymers or epoxy may reduce these tendencies.

4. RUBBER

Rubber also has the problems of extraction of drug ingredients and leaching of container ingredients described for plastics.

The use of neoprene, butyl or natural rubber, in combination with certain epoxy, Teflon or varnish coatings reduces drug-container interactions.

PRESERVATIVES

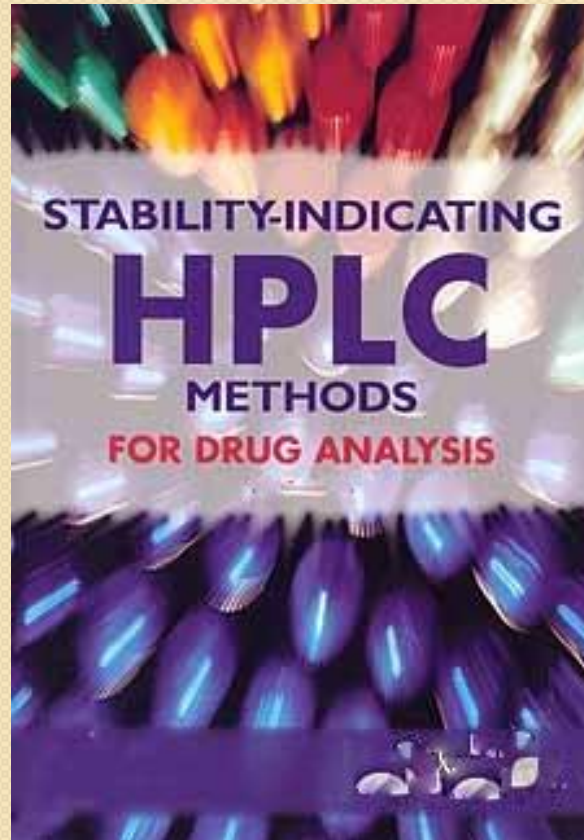
PRESERVATIVES USED IN PHARMACEUTICAL PREPARATIONS

The following gives a list of usual preservatives used in pharmaceutical preparations:

PREPRATION	PRESERVATIVE	CONCENTRATION % w/v
INJECTIONS	Phenol	0.5
	Cresol	0.3
	Chlorocresol	0.1
	Phenylmercuric nitrate	1.0
EYE DROPS	Phenyl mercuric nitrate or acetate	0.002
	Chlorhexidine acetate	0.01
	Benzalkonium chloride	0.01

MIXTURES	Chloroform	0.25
	Benzoic acid	0.1
	Methyl paraben	0.1
	Sulphur dioxide	400 parts/10 ⁶
TABLETS	Methylparaben	0.1
CREAMS	Parabens	0.1-0.2
	Chlorocresol	0.1
	Dichlorobenzyl alcohol	0.05-0.2
	Cetyltrimethyl ammonium Bromide	0.01-0.1
	Phenylmercuric nitrate	0.001
		34

STABILITY INDICATING ASSAYS



STABILITY IDENTIFYING ASSAYS

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

➤ STABILITY INDICATING ASSAY DEVELOPMENT

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

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

➤ OBTAINING A REPRESENTATIVE SAMPLE

Pure drug compound degrades into toxic compound.

Formulation ----

degradation → drug (toxic) + inert (non-toxic).

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 & greater 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 collect sample at interval of 1,2,4,8,24 hrs.

- Similarly reaction is quenched in BASE .
- FOR OXIDATION (with peroxides) 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.

SEPERATION

REVERSE PHASE CHROMATOGRAPHY is the method of choice for stability indicating assays because the samples are generated in aqueous solutions . (non polar stationary phase).

- We should choose gradient elution for sample screening.
- Most commonly used solvent type are - acetonitrile , 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 .
- Column temperature (35—50°C).
- Core set of experiments should be runs for each sample.
- After the screening runs are completed .
- Now match the peaks between runs so that each compound can be tracked as the conditions change.
- Although each sample might contain only 4 or 6 significant degradants ,different 38degradation conditions can produce some of the same compounds in addition to unique degradation.

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THANK YOU