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Role of Chemical and Analytical Reagents in Colorimetric Estimation of Pharmaceuticals-A Review

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Abstract
The aim of present work is to find out a simple, specific, colorimetric / spectrophotometric method developed for the detection of different pharmaceutical dosage forms using analytical and chemical reagents in bulk and pharmaceutical formulation. The developed methods have been stastically validated for application in pharmaceutical quality control laboratory. The proposed method was ascertained by actual determination of fixed concentration of the drug with in Beers range and finding out the absorbance by the proposed method with suitable reagent. Hence in present work different reagents, their properties, uses, mechanism of action and applications are enlisted.

Introduction
Substances used for the detection, identification, analysis, etc. of chemical, biological, or pathologic processes or conditions. Indicators are substances that change in physical appearance, e.g., colour, at or approaching the endpoint of a chemical titration, e.g., on the passage between acidity and alkalinity. Reagents are substances used for the detection or determination of another substance by chemical or microscopical means, especially analysis. Types of reagents are precipitants, solvents, oxidizers, reducers, fluxes, and colorimetric reagents.[1] Reagent is a "substance or compound that is added to a system in order to bring about a chemical reaction, or added to see if a reaction occurs."[1] In organic chemistry, reagents are compounds or mixtures, usually composed of inorganic or small organic molecules that are used to effect a transformation on an organic substrate. Examples of organic reagents include the Collins reagent, Fenton's reagent, and Grignard reagent. There are also *analytical reagents* which are used to confirm the presence of another substance. [1]

Advantages:

- Analytical reagents are very cheaper to use
- Time consuming is less
- More reliable
- Compatible with most of drugs
- No harmful reactions occurs
- Easy of preparation

List Of Reagents

Bratton-Marshall Reagent:

It is chemically *N*-1-naphthyl ethylene diamine dihydrochloride. It is widely used for the determination of drugs and pharmaceutical containing free primary aromatic amino group. At present, it is employed for the determination of sulpha drugs and local anaesthetics.[2]

Chemical Name: N-(1-Naphthyl)ethylenediamine dihydrochloride

Synonyms: Monomethanolate;
Bratton's reagent
Marshall's reagent
Bratton-marshall reagent

Molecular Formula: C12H16Cl2N2
Formula Weight: 259.17

Structure:

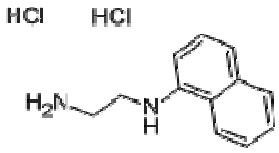

Figure.1 Shows the Structure of Bratton Marshall reagent

Mechanism of Action:
The primary aromatic amino group is first diazotized with sodium nitrite and hydrochloric acid. The excess nitrous acid (HNO_2) is neutralized by treating with ammonium sulphamate reagent. Finally, the diazonium ion is allowed to couple with BM reagent to produce a highly colored azodye complex measured at 550 nm. [2]
Reaction:

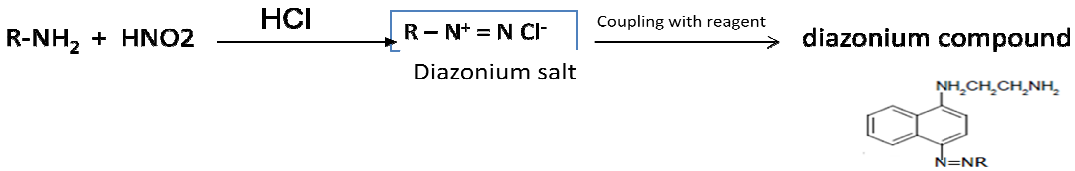

Figure.2 Mechanism of Bratton Marshall reagent

Preparation of Reagent: Dissolve 100 mg of *N*-1-naphthyl ethylene diamine dihydrochloride in 100 ml of a mixture of seven parts of acetone and three parts of water

Usage: Coupling agent for the spectrophotometric determination of amino phenols, phenylenediamines, dinitroanilines, chloroanilines, thiols, and sulfonamides.1,2,3

General Description: White to light tan or gray crystalline solid or off-white powder.

Air & Water Reactions: N-(1-Naphthyl) ethylenediamine dihydrochloride is hygroscopic. Slightly soluble in water.

Reactivity Profile: N-(1-Naphthyl) ethylenediamine dihydrochloride may decompose on exposure to light. N-(1-Naphthyl) ethylenediamine dihydrochloride is incompatible with acids, acid chlorides, acid anhydrides and oxidizing agents.

Composition of Bratton Marshall reagent:
N-[1-Naphthylyl] ethylenediaminedihydrochloride. 0.05%w/v
Ammonium sulphamate 0.5%w/v
Sodium Nitrite 0.5%w/v
Hydrochloric Acid 1.5 M
Applications : The Bratton-Marshall reagent is one of the real land-marks in the development of drug metabolism and pharmacokinetics, coming at a time when highly sensitive and specific analytical procedures were desperately needed for the measurement of drug concentrations in the body. Examples of its applications are taken from early work in the mid-40's and 50's in the Parke-Davis Research Laboratories, extending from primary aromatic amines (e.g., sulphonamides), to p-nitro phenyl compounds that must first be reduced to amines (e.g., chloramphenicol), and to phenyl derivatives that must be nitrated on a microgram scale and then reduced to aryl amines (e.g., phenytoin). The development and use of separation techniques such as liquid/liquid counter-current partition and paper chromatography is described. Emphasis is placed upon continued, progressive improvement in the basic assay procedures over long periods of time [2]

2. 2, 6-Dichloroquinone-4-Chloroimide
Synonyms [3]

- 2,6-dichloro-*p*-benzoquinone-4-chloroimine;
- *N*,2,6-trichloro-*p*-quinoneimine;
- 2,6-dichloroquinone chloroimide
- *N*,2,6-trichloroquinoneimine;
- *N*,2,6-trichlorobenzoquinone imines
- Gibbs reagent

Iupac name: 2,6-Dichloro-4-(chloroimino)cyclohexa-2,5-dienone.
Molecular formula: $C_6 H_2Cl_3 N O$
Appearance: Yellow crystals
Structure:

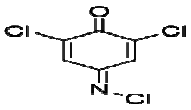

Figure.3 Shows Structure of Gibbs reagent

Use: In determination of phenols, for detection of antioxidants, primary and secondary aliphatic amines, secondary and tertiary aromatic amines, aromatic hydrocarbons, pharmaceuticals, phenoxyacetic acid herbicides, etc [3]

Preparation:

- Spray with a solution of 3% 2,6-dibromo-N-chloro-p-benzoquinone imine in toluene or methanol.
- Spray with a freshly prepared 0.5-2% solution of 2,6-dichloroquinone-4-chloroimide in ethanol (reagent stable for 3 weeks if refrigerated).

Principle And Mechanism of Action:

- When Phenolic compounds reacts with Gibbs reagent ,coupling reaction may occur.
- The first step of reaction is formation of the corresponding quinine imines.
- Quinone imines are condensation products of quinone chlorimines with phenols in aqueous alkaline media.Imides portion of Gibbs reagent reacts with phenolic compounds gives corresponding products coupling with nucleophilic sites by the elimination of chlorine[3]

Amines: Gibbs reagent couples with amine by elimination of HCL and results in coloured complex which is measured at characteristic maximum wavelength.

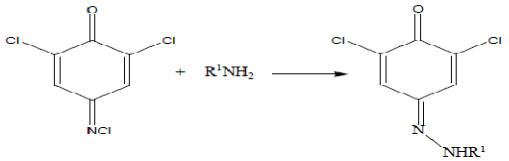

Figure.4 Reaction of Gibbs reagent with Amines

Phenols: when a phenol reacts with Gibbs reagent, reagent couples at Para position.

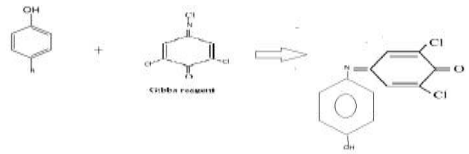

Figure.5 Reaction of Gibbs reagent with Phenols [4]

Estimation of Various Drugs Using 2, 6-Dichloroquinone-4-Chloroimide [5]

1. Methyldopa: Aliquots of 0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml of the stock solution are transferred to 10ml volumetric flasks followed by addition of 2ml, 1.5ml, 1.0ml, 0.5ml and 0ml water to the five 10ml volumetric flasks respectively. 1ml of sodium acetate buffer and 1ml of Gibb's reagent was added to each flask. The solutions are allowed to stand for one hour and then make it up to 10ml with water. A blank solution is prepared in the same way using 2.5ml water instead of 2.5 ml standard stock solution of methyldopa. The absorbance of each solution is measured at 400nm against blank. A calibration graph is constructed by plotting the absorbance against the concentration of the drug. Similarly the absorbance of sample solution is measured and the amount of drug methyldopa is determined by referring to the calibration curve.

2. Captopril: Aliquot volumes of Captopril (pure sample) covering the working concentration range (10-50 µg/mL) were transferred into a series of 10 ml volumetric flasks. 5 mL ± 1 mL of DMSO was added, followed by addition of 0.7 ± 0.1 mL of DCQ. The flasks were completed to the mark with distilled water and allowed to cool. The absorbance of the resulting solution was measured at 443 nm against a reagent blank prepared simultaneously. Similarly the absorbance of sample solution is measured and the amount of captopril is determined by referring to the calibration curve.

- It is a very good reagent for Vitamin B_6.
- It is used for the identification of un-substituted and p-alkoxy phenols. .
- It is used to identify octapamine in mammals.

- It is used for the detection of anti oxidants, 1^o,2^o aliphatic amines, 2^o,3^o aromatic amines , aromatic hydrocarbons , phenoxy acetic acid herbicides.
- .Pyridoxine – blue colour -650nm.

3. Dimedone

Dime done is a cyclic diketone used in organic chemistry to determine whether a compound contains an aldehyde group. Cyclohexanediones in general can be used as catalysts in the formation of transition-metal complexes. Other uses include applications in colourimetry, crystallography, luminescence and spectrophotometric analysis. It can also be used for chemistry involving organic compounds of low electrical resistance.

Systematic name : 5,5-Dimethyl-1,3-cyclohexanedione

Molecular Formula: $C_8H_{12}O_2$

Synonyms: Cyclomethone,
5,5-dimethyl-1,3-cyclohexanedione,
Dimethyldihydroresorcinol,
Methone

Structure:

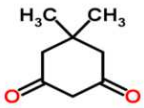

Figure.6 Shows Structure of Dime done

Synthesis: Dime done is prepared from mesityl oxide and diethyl malonate.

Physical properties: Dime done usually comes in the form of white crystals. It is stable under ambient conditions and soluble in water, as well as ethanol and methanol. It has a melting point range of 147–150 °C (420–423 K.)

Applications: Cyclohexanediones can be applicable for the industry of

- Transition-metal complex catalyst chemistry
- Luminescence chemistry and spectrophotometric analysis
- Organic synthesis
- Crystallography and Crystal Chemistry
- Organic low electrical resistance Chemistry
- Colorimetry
- Dime done is used for the colorimetric determination of paracetamol and oxyphenbutazone both in pure form and in their tablets. Paracetamol and oxyphenbutazone are first nitrated and the nitroderivatives produced are extracted into ethyl acetate. Dime done is added in the presence of triethylamine and a highly coloured complex is formed. The concentration ranges adhering to Beer's law are 0.01 - 0.1 mg/ml for oxyphenbutazone and 0.03 - 0.1 mg/ml for paracetamol.

4. Folin Ciocalteu Reagent

The folin ciocalteu reagent / folin phenol reagent/folin denis reagent also called the gallic acid equivalence method (GAE) , is a mixture of phosphomolybdate & phosphotungstate used for the colorimetric in vitro assay of phenolic & polyphenolic antioxidants .It is named after ottofolin , vintila ciocalteu, & willey glover denis.[6]

Appearance:

Clear bright yellow solution.

Storage: FC should be stored tightly capped at room temperature. The reagent can be diluted with deionised water.

Method of preparation: Dissolve 10g of sodium tungstate & 2.5g of sodium molybdate in 70ml of water add 5ml 85% Phosphoric acid & 10ml con. Hydrocholric acid reflux for 10 hrs. Add 15g lithium sulphate 5ml water & 1 drop bromine. Reflux for 15min .cool to room temperature and bring to 100ml with water.[6]

Molecular formula:

$3H_2O \cdot P_2O_5 \cdot 13WO_3 \cdot 5MoO_3 \cdot 10H_2O$

$3H_2O \cdot P_2O_5 \cdot 14WO_3 \cdot 4MoO_3 \cdot 10H_2O$

Uses: Amino, phenolic groups

Principle: "Reduction"

When FC reagent reacts with drug in presence of reducing agents like $SnCl_2$, Ascorbic acid hydrazine, probably drug effects reduction of 1 or more oxygen atoms from tungstate or molybdate in the F-C reagent, there by producing one or more possible reduced species which have characteristic intense blue colour.[6]

Reaction:

Phosphomolibdo tungstate $\xrightarrow{\text{reducing agents}}$ Molybdic blue or tungstic blue

Reducing agents: $Sncl_2$, Ascorbic acid, Hydrazine, Feso4, Thiourea, p-aminophenol hydrochloride.

Estimation of Various Drugs Using Folin Ciocalteu Reagent[6]

- The most common usage of this reagent is in the Lowry method for determining protein concentration. In this method, protein is pre-treated with copper(II) in a modified biuret reagent (alkaline copper solution stabilized with sodium potassium tart rate). Addition of Folin & Ciocalteu's phenol reagent generates chromogens that give increasing absorbance between 550 nm and 750 nm. Normally, absorbance at the peak (750 nm) is used to quantify protein concentrations between 1-100 μg/ml while absorbance at 550 nm is used to quantitate higher protein concentrations. In the absence of copper, color intensity would be determined primarily by the tyrosine and tryptophan content of the protein, and to a lesser extent by cysteine, and histidine. Copper(II) enhances color formation by chelation with the peptide backbone, thus facilitating the transfer of electrons to the chromogens.
- Spectophotometric determination of Dastinib at 765nm
- Determination of total proteins in gemo therapeutic preparations with the Folin-Ciocalteu reagent which produce blue colour measuring with the colorimeter at a wave-length of 750 nm.
- Spectrophotometric determination of cefadroxil in dosage forms through the reaction with Folin-Ciocalteu reagent in presence of NaOH and stannous chloride form a blue coloured chromogen which was measured At 970 nm.

5. Froehde Reagent

The **Froehde reagent** is used as a simple spot-test to presumptively identify alkaloids, especially opioids, as well as other compounds. It is composed of a mixture of molybdic acid or sodium molybdate and hot, concentrated sulfuric acid, which is then dripped onto the substance being tested.[7] A presumptive test for opioids. Froehde's reagent consists of 0.5 gram of sodium molybdate (Na_2MoO_4) dissolved in 100 ml of concentrated sulphuric acid. LSD gives a blue-green colour, heroin gives purple to olive green, and mescaline gives a greenish colour. [8]

6. Hydroxylamine

Hydroxylamine is an inorganic compound with the formula NH_2OH. The pure material is a white, unstable crystalline, hygroscopic compound. However, hydroxylamine is almost always provided and used as an aqueous solution. It is used to prepare oximes, an important functional group. It is also an intermediate in biological nitrification. The oxidation of NH_3 is mediated by the enzyme hydroxylamine oxidoreductase (HAO).

Structure:

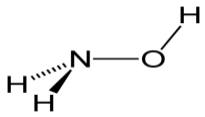

Figure.7 Shows Structure of Hydroxylamine

IUPAC name: Hydroxylamine

Other names: Aminol, Azanol, Hydroxyamine, Hydroxyazane, Hydroxylazane

Uses: Hydroxylamine and its salts are commonly used as reducing agents in myriad organic and inorganic reactions. They can also act as antioxidants for fatty acids. Some non-chemical uses include removal of hair from animal hides and photography developing solutions. The nitrate salt, hydroxyl ammonium nitrate, is being researched as a rocket propellant, both in water solution as a monopropellant and in its solid form as a solid propellant. This has also been used in the past by biologists to introduce random mutations by switching base pairs from G to A, or from C to T. This is to probe functional areas of genes to elucidate what happens if their functions are broken.. Hydroxylamine can also be used to highly selectively cleave asparaginyl-glycinepeptide bonds in peptides and proteins. It also bonds to and permanently disables (poisons) heme-containing enzymes. It is used as an irreversible inhibitor of the oxygen-evolving complex of photosynthesis on account of its similar structure to water. Simple, accurate and precise colorimetric method for the determination of simvastatin and lovastatin in tablets is described. The method is based on the reaction of simvastatin or lovastatin with hydroxylamine in alkaline medium to form the corresponding hydroxamic acid derivatives which, on treatment with ferric ion in acid medium, yield highly colored ferric-chelate complex with maximum absorption at 513nm

7. Molybdenum Blue

Molybdenum blue is a term applied to

- reduced heteropolymolybdate complexes, polyoxometalates containing Mo(V), Mo(VI), and a hetero atom such as phosphorus or silicon
- reduced isopolymolybdate complexes, polyoxometalates containing Mo(V), Mo(VI) formed when solutions of Mo(VI) are reduced
- a blue pigment containing molybdenum(VI) oxide The "heteropoly-molybdenum blues", are used extensively in analytical chemistry and as catalysts. The formation of "isopropyl-molybdenum blues" which are intense blue has been used as a sensitive test for reducing reagents. They have recently been

shown to contain very large anionic species based on the so-called "big wheel" containing 154 Mo atoms, with a formula $[Mo_{154}O_{462}H_{14}(H_2O)_{70}]^{14}$.

Principle: Heteropoly-molybdenum blues **.** The first heteropoly molybdate and first heteropolymetallate, yellow ammonium phosphomolybdate, $(NH_4)_3PMo_{12}O_{40}$ was discovered by Berzelius in 1826. The phosphorus atom in the anion is termed the hetero-atom; other hetero-atoms are silicon and arsenic. The heteropoly-molybdenum blues have structures based on the Keg gin structure. The blue colour arises because the near-colourless anion, such as the phosphomolybdate anion, $PMo_{12}O_{40}^{3-}$, can accept more electrons (i.e. be reduced) to form an intensely coloured mixed-valence complex. This can occur in one electron or two electron steps. The reduction process is reversible and the structure of the anion is essentially unchanged.

The structure of the anion, $PMo^{V}_{4}Mo^{VI}_{8}O_{40}^{7-}$, has been determined in the solid state and is a β-isomer (i.e. with one of the four groups of edge-shared octahedra on the α-Keg gin ion rotated through 60 degrees) Similar structures have been found with silicon, germanium or arsenic hetero-atoms.

The intense blue colour of the reduced anion is the basis for the use of heteropoly-molybdenum blues in quantitative and qualitative analytical techniques. This property is exploited as follows:

- the sample to be analysed is reacted to produce the reduced blue heteropoly-molybdate in order to:
  - detect the presence of a hetero atom in e.g. a spot test
  - measure the amount of a hetero atom present in the sample colorimetrically
- the sample is added to a solution of the near colourless, un-reduced complex in order to:
  - detect the presence of a reducing compound e.g. a reducing sugar such as glucose
  - measure the amount of a reducing compound in a two step procedure

Uses in quantitative analysis:

Colorimetric determination of P, As, Si and Ge : The determination of phosphorus, arsenic, silicon and germanium are examples of the use of heteropoly-molybdenum blue in analytical chemistry. The following example describes the determination of phosphorus. A sample containing the phosphate is mixed with an acid solution of Mo^{VI}, for example ammonium molybdate, to produce $PMo_{12}O_{40}^{3-}$, which has an α-Keg gin. This anion is then reduced by, for example, ascorbic acid or $SnCl_2$, to form the blue coloured β-keg gin ion, $PMo_{12}O_{40}$ The amount of the blue coloured ion produced is proportional to the amount of phosphate present and the absorption can be measured using a colorimeter to determine the amount of phosphorus. Examples of procedures are:

- The analysis of phosphate in sea water.
- Standard methods for determining phosphorus and silicon content of metals and metal ores.
- the determination of germanium and arsenic
- The comparison of the measured absorption against readings taken for analyses of standard solutions means that a detailed understanding of the structure of the blue complex was unnecessary.This colorimetric method is not effective when comparable amounts of arsenate are present in solution with phosphate. This is due to the strong chemical likeness of arsenate and phosphate. The resultant molybdenum blue for arsenate, using the same procedure, does produce a slightly different spectral signature, however.

Colorimetric determination of glucose The Folin-Wu and the Somogyi-Nelson methods are both based on the same principles. In the first step glucose (or a reducing sugar) is oxidised using a solution of Cu(II) ion which in the process is reduced to Cu(I). In the second step the Cu(I) ions are then oxidised back to Cu(II) using a colourless hetero-polymolybdate complex, which is, in the process, reduced to give the characteristic blue colour. Finally the absorption of the hetero-poly molybdenum blue is measured using a colorimeter and compared to standards prepared from reacting sugar solutions of known concentration, to determine the amount of reducing-sugar present. The Folin-Wu method uses a reagent that contains sodium tungstate. The exact nature of the blue complex in this procedure is not known. The Somogyi-Nelson method uses an arsenomolybdate complex formed by the reaction of ammonium molybdate, $(NH_4)_6 Mo_7O_{24}$, with sodium arsenate, Na_2HAsO_7.

Colorimetric determination of some drugs containing o-hydroquinone Some drugs that contain an o-hydroquinone group react with phosphomolbydic acid to give the heteropoly-molybdenum blue colour. Micro quantities of the drugs can be determined.

8. **3-Methyl-2-Benzothiazoline Hydrazone**

Synonyms:

- Mbth
- Mbth monohydrate
- Sawicki's reagent
- Besthorn's hydrazone
- Mbth hydrochloride hydrate
- 3-Methyl-2-benzothialinone [9]

Structure:

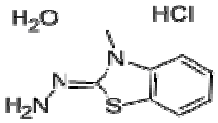

Figure.8 Shows Structure of MBTH reagent

Uses: Phenols, Aldehydes, Aromatic amines.
Principle:"Oxidation followed by coupling".
In general MBTH undergoes oxidative coupling reaction catalysed by iron .Under reactionConditions MBTH loses 2 electrons and one proton forming an electrophilic intermediate,Which is the active coupling species .This intermediate undergoes electrophilc substitutionWith the phenols, amines, aldehydes, to form coloured product. [10]
Reaction:

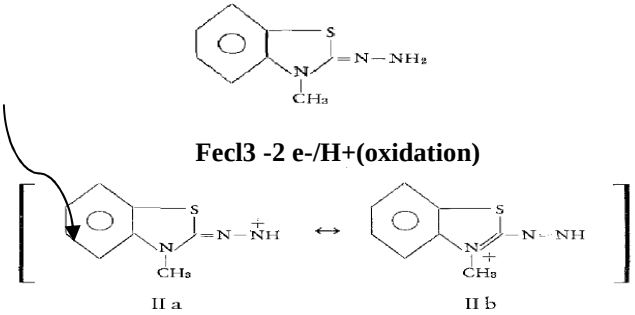

Figure .9 Mechanism of MBTH reagent

Estimation of Various Drugs Using Mbth Reagent [11]

1. Acyclovir

Procedure: MBTH (1 ml) was transferred into a series of 10-ml volumetric flasks. Aliquot volumes of acyclovir standard solution was added so that the final concentration was in the range of 20-200 mg/ml, then 1 ml of ferric chloride solution was added. This solution was mixed and allowed to stand for 20 min. The volume was adjusted to the mark with water. The absorbance was measured against a reagent blank (which contains all reagents except acyclovir) at 616 nm. The absorbance versus the final concentration was plotted to get the calibration curve, or to derive the regression equation.

2. Ceftazidime

Procedure: Aliquots of the working standard solution of the drug (0.2–1.0 mL) (100 mg ml^{-1}) were transferred into 10-mL calibrated flasks. To each aqueous solution of $FeCl_3$ (1.5 mL, $3*10^{-2}$ mol L^{-1}), an aqueous solution of MBTH (1.0 mL, $8.6 * 10^{-3}$ mol L^{-1}) was added. The solutions were swirled and allowed to stand for 5 min. Then HCL (1.0 mL, $1*10^{-2}$ mol L^{-1}) was added and made up to the mark with water. The absorbance was measured at 628 nm against the corresponding reagent blank and calibration graph was constructed.

3. Ganciclovir:

Procedure: Fresh aliquots of Ganciclovir ranging from 0.5 to 2.5 mL (1 µg/mL-1000µg/mL) were transferred into a series of 10 mL volumetric flasks to provide final concentration range of 50 to 250 µg/mL. To each flask 1ml of aqueous Ferric chloride (1%) solution and 1 ml of MBTH reagent (0.5% in distilled water) were added. The solution in each tube were made up to the mark with distilled water. The absorbance of bluish green colored chromogen was measured at 611.6 nm against the blank. The amount of Ganciclovir present in the sample solution was computed from its calibration curve

4. Cefadroxil

Procedure: To a series of 25-ml calibrated flasks, containing aliquots of CFL (0.5-3.0 ml, 50 µg/ml), 2.0 ml portions of MBTH solution were added and kept for 2 min at room temperature. Then 2.0 ml of Ce(IV) solution was added, kept for 15 min and diluted to the mark with water. The absorbencies were measured within 45 min at 410 nm against a reagent blank. The CFL concentration was read from a calibration graph prepared under identical conditions.

9. Para – Dimethyl Amino Benzaldehyde

para-Dimethyl aminobenzaldehyde is a bifunctional aromatic skeleton possessing the aldehyde\ (CHO) *Para* to an activating substituent dimethyl amino group [-N(CH3)2].

Structure:

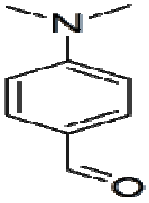

Figure.10 Shows Structure of PDAB reagent

Synonyms:

- 4- (dimethylamino) benzaldehyde ,
- *p*-(dimethylamino)-benzaldehyde,
- Ehrlich's Reagent;
- *p*-Formyl-N,N dimethyl aniline;
- p-DAB;
- N,N-Dimethyl-4-aminobenzaldehyde
- Wasickys reagent.

Principle: "formation of condensation products referred as Schiff's base".

Uses: *para*-Dimethylaminobenzaldehyde is an organic compound containing amine and aldehyde moieties which is used in Ehrlich's reagent for determination of hydrazine and Kovac's reagent for microbiology's in dole test

Estimation of Various Drugs Using Para – Dimethyl Amino Benzaldehyde [13]

- 1 Urobilinogen determination
- 2 Hydrazine determination

Urobilinogen determination

- It is used in Ehrlich's reagent, which may be used as a stain in thin layer chromatography, or as a reagent to detect urobilinogen in fresh, cool urine.
- It can be used to detect the presence of in dole alkaloids. Not all in dole alkaloids give a colored adduct as result of steric hindrance which does not allow the reaction to proceed.

Hydrazine determination

- *P*-Dimethylaminobenzaldehyde reacts with hydrazine to form an azo-dye, which has a distinct yellow colour. It is therefore used for spectrophotometric determination of hydrazine in aqueous solutions at 457 nm.
- Ehrlich's reagent, also known as the "DMAB test" is used as a simple spot-test to presumptively identify alkaloids. It is prepared by dissolving 2.0 g of p–dimethylaminobenzaldehyde (DMAB) in 50 mL of 95% ethanol and 50 mL of concentrated hydrochloric acid. It is best prepared fresh.
- It is primarily used to identify in doles, and a consumer kit can be bought for the testing of psychoactive in dole containing drugs like LSD and psilocybin.
- The ehrlich reagent is similar to a number of other in dole tests
- The van Urk reagent which uses DMAB, sulphuric acid and an oxidant. .

Analytical Applications

The analytical applications of DMAB comprise its utilization for the determination of a wide range of substances notably among which are pharmaceuticals (inorganic and organic), bioactive substances and nanomaterials. In the applications of DMAB as an analytical reagent, use is made of its peculiar properties of forming condensation products, the ability of its aldehyde moiety being reduced to the alcohol or oxidized to the carboxylic acid.

Chromatographic methods.

A method is described for the HPLC determination of phenylpropanolamine (PPA) based on pre column derivatization with DMAB and elution from Phenomenex C-18 column with methanol–water and detection by spectrophotometer at 418 nm. Linear calibration was obtained with 9.4–46.9 μg mL−1 with a detection limit of 4.7 ng mL−1. Vitamin B12 and rifampicin when present together with PPA separated completely and could be determined simultaneously.

Spectrophotometric methods

1. DMAB has been adopted solely or in combination with some reagents for the spectrophotometric analyses of pharmaceuticals. A simple, accurate and sensitive spectrophotometric method has been developed and validated for determination of H2-receptor antagonists: cimetidine, famotidine, and nizatidine and ranitidine hydrochloride. The method was based on the oxidation of these drugs with cerium (IV) in presence of perchloric acid and subsequent measurement of the excess Ce (IV) by its reaction with DMAB to give a red coloured product (λmax at 464 nm).
2. Secnidazole has also been determined spectrophotometrically through Schiff base formation with DMAB with measurement made at 494 nm yielding good accuracy and reproducibility.

10. 4-(Dimethylamino) Cinnamaldehyde

DMACA is an histological dye used to detect in dole production in cells. It is used for the rapid identification of bacteria containing tryptophanase enzyme systems. It is also particularly useful for localization of proanthocyanidins compounds in plants, resulting in a blue staining. It has been used for grapevine fruit or for legumes foliage histology.

Structure:

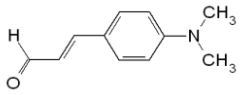

Figure.11Shows Structure of DMACA reagent

Principle: Certain amines condense with various aldehydes in strongly acidic media to give products that are ox disable to give color. Among many, the following aldehydes are widely used. They are *p*-dimethylaminobenzaldehyde, vanillin, formaldehyde, benzaldehyde, salicyaldehyde, paraldehyde, *p*-acetyl aminobenzaldehyde, *m*- and *p*-nitrobenzaldehyde, etc.

The reaction with aromatic amines produces Schiff bases.

Chemical Name:	4-(Dimethylamino)cinnamaldehyde
Synonyms:	DMACA Reagent 4-Dimethylcinnamaldehyde P-Dimethylaminocinnamaldehyde 4-Dimethylaminocinnamic aldehyde
Molecular Formula:	C11H13NO
Formula Weight:	175.23

Mechanism of action: The mechanism of aldehydes which condenses with the aromatic amines involves the condensation of the aldehydes to release the oxygen molecule and then it combines with the amine group to form the yellow Schiff's base in the presence of acidic medium such as HCl or H_2SO_4

Reaction:

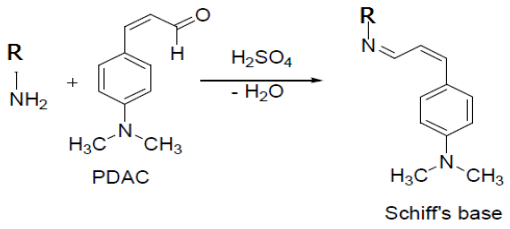

Figure.12 Mechanism of DMACA reagent

Uses:

Used for identification of Phenols, aldehyde, 1^0aromatic amines, poly hydroxyl compounds, anti pyrene, active methylene group compound

Applications:

Used for the colorimetric determination of cysteine and cystine, but does not react with other amino acids except cyst amine (reacted in methanol at 60°C for 2 hours in the presence of sulphuric acid as a catalyst; absorbance measured at 587 nm). Can be used to detect p-aminohippuric acid with the reaction carried out in ethanol rather than dilute acids. Indole Spot Reagent is used to detect the presence of in dole, which is one of the degradation products of the bacterial metabolism of tryptophan. [17]

- Estimation of sumatriptan succinate in bulk and formulations by visible spectrophotometry using Para dimethyl amino cinnamaldehyde(PDAC) in the presence of sulphuric acid in non aqueous medium and formed purple red colored condensation products with an absorption maximum of 565nm.
- Determination of dulcin prepared in methanol, acid used is p-toluene sulphonic acid. Wavelength of absorption maximum was 520nm.
- Determination of indomethacin prepared in isopropanal. Color formed at 560nm, acid used was sulphuric acid.
- Determination of mefanemic acid using sulphuric acid. Condensation product is bluish green color at 660nm.

11. PHOSPHOMOLYBDIC ACID

Phosphomolybdic acid, also known as dodeca molybdophosphoric acid or PMA is a component of Masson's trichrome stain. It is a yellow-green compound, freely soluble in water and polar organic solvents such as ethanol. It is used as a reagent in thin layer chromatography for staining phenolics, hydrocarbon waxes, alkaloids and steroids

Conjugated, unsaturated compounds reduce PMA to molybdenum blue. The colour intensifies with increasing number of double bonds in the molecule being stained Phosphomolybdic Acid is primarily used in spectrophotometric determination of phosphate ions. It is also component in the 3-color histology stain called Masson's trichrome; employed in the differentiation step. It is one of the solutions fixed tissues are immersed in prior to subsequent staining.

Use: Phosphomolybdic Acid is also used as reagent for simple and rapid colorimetric determination of phenothiazine derivatives.

Appearance:	Liquid
Synonyms:	PMA
Molecular Formula:	HMoO2P
Molar Mass	159.92 g/mol

Structure:

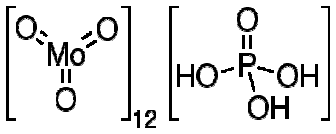

Figure.13 Shows Structure of Phosphomolybdic acid

12. Simon's Reagent

Simon's reagent is used as a simple spot-test to presumptively identify alkaloids as well as other compounds. It reacts with secondary amines like MDMA and methamphetamine to give a blue solution.[14]

Chemistry: The reagent is composed of a mixture of sodium nitroprusside, sodium carbonate and acetaldehyde, which is dripped onto the substance being tested. The amine and acetaldehyde produce the enamine, which subsequently reacts with sodium nitroprusside to the imine. Finally, the immonium salt is hydrolysed to the bright cobalt-blue Simon-Awe complex.

Uses [15]: The primary use of this reagent is for detecting secondary amines, such as MDMA and methamphetamine, and is typically used after the mecke or marquis reagents to differentiate between the two mentioned and amphetamine or MDA

Simon reagent with acetone:

- ✓ As expected, this variant of Simon reagent gives no reaction with the secondary amines ephedrine and pseudoephedrine, while a light purple or light pink colour develops with the two nor-derivatives nor ephedrine and nor pseudoephedrine. The colours are weak and develop slowly (3 to 5 minutes).
- ✓ Chloropseudoephedrine gives no colour, while the tertiary amine N-methyl ephedrine gives a distinct light orange colour with this variant of the Simon reagent.

Simon reagent with acetaldehyde:

- ✓ Ephedrine reacts with the expected blue colour, but the intensity of the colour is weak as compared to methamphetamine and to other secondary amines such as piper dine. The full development of the colour requires 5 to 10 minutes. This may be due to the effect of vicinal hydroxyl functional group. Chloropseudoephedrine also results in a blue colour. However, this colour is unstable and changes into grey after a few minutes.
- ✓ Nor ephedrine and N-methyl ephedrine react with distinct colours: olive green and pale pink, respectively. The keto-analogues cathinone and methcathinone react with unstable initial colours. The brown solution of cathinone subsequently turns into a white precipitate, while the initial greyish-blue colour of methcathinone turns into brown within about 5 minutes. These colour sequences may indicate the decomposition of the two compounds under alkaline conditions of the Simon reaction. It is known that both cathinone and methcathinone are rather unstable as free bases in alkaline conditions giving rise to a series of decomposition products (benzaldehyde, ethylamine, phenylpropanedione and a pyrazine dimer).
- ✓

13. 2, 3, 5, Triphenyl Tetrazolium Salt

Chemical name: 2,3,5-Triphenyltetrazolium chloride[16]

Synonyms:

- ➢ Urocheck
- ➢ TTC(TTZ);

- uroscreen;
- vita stain;
- tetrazole red

Molecular formula: C19H15ClN4
Weight: 334.8

Structure:

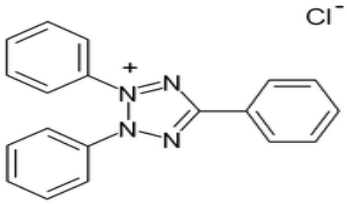

Figure.14 Shows Structure of TTC reagent

Solubility: water, ethanol and acetone soluble, Ether insoluble
Colour: white

Principle: Triphenyl tetrazolinum chloride TTC is a red ox indicator commonly used in biochemical experiments especially to indicate cellular respiration In the TTC assay (also known as TTC test or tetrazolium test), TTC is used to differentiate between metabolically active and inactive tissues. The white compound is enzymatic ally reduced to red TPF (1,3,5-triphenylformazan) in living tissues due to the activity of various dehydrogenases (enzymes important in oxidation of organic compounds and thus cellular metabolism), while it remains as white TTC in areas of necrosis since these enzymes have been either denatured or degraded. For this reason, TTC has been employed in autopsy pathology to assist identification of post mortem myocardial infarctions. Healthy viable heart muscle will stain deep red from the cardiac lactate dehydrogenase; while areas of potential infarctions will be more pale [16].
Note: TTC is somewhat heat and light
Reaction:

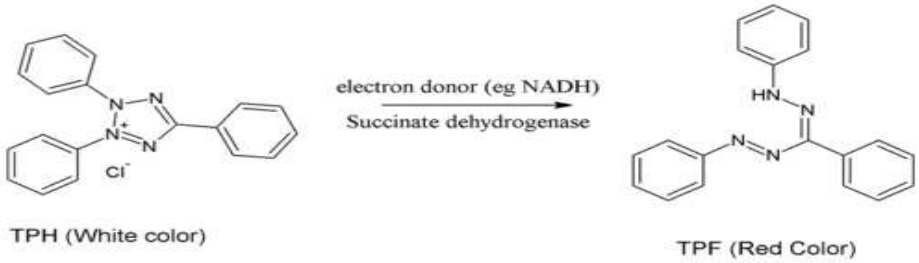

Figure.15 Mechanism of TTC reagent

Estimation of Various Drugs Using 2, 3,5triphenyltetrazolium Chloride [17]
Colorimetric determination of catecholamine's by 2, 3,5triphenyltetrazolium chloride:

- A convenient spectrophotometric method was developed for the determination of epinephrine, levarterenol, isoproterenol, and methyldopa by reduction of 2,3,5-triphenyltetrazolium chloride and subsequent measurement of the formazan at 485 nm.
- With absolute alcohol as the solvent, maximum color absorption was attained in 30 min at 25 degrees in the presence of 0.1 N KOH. Evidence is provided to account for the reduction of the tetrazolium salt at the expense of the epinephrine catechol moiety.
- In addition to the considerably high values of the molar absorptivities of the chromogen formed, ideal adherence of the color absorption to the Beer-Lambert law permitted a sensitive micro determination of these catecholamine's in both pure forms and pharmaceutical formulations.
- The tetrazolium interaction was selective.
- No interference was encountered from common catecholamine antioxidants, adjutants, or noncatechol degradation products

14. Zwikker Reagent
The **Zwikker reagent** is used as a simple spot-test to presumptively identify barbiturates

Structure:

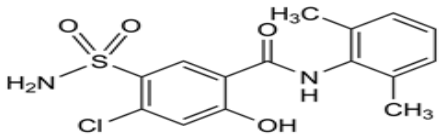

Figure.16 Shows Structure of Zwikker reagent

Preparation:

It is composed of a mixture of two solutions. Part A is 0.5 g of copper (II) sulphate in 100 ml of distilled water. Part B consists of 5% pyridine (v/v) in chloroform.

Uses:

- One drop of each is added to the substance to be tested and any change in colour is observed.
- The test turns Phenobarbital, pentobarbital and secobarbital light purple. Tea and tobacco turn yellow-green.
- The test's lack of specificity and tendency to produce false positives means it is not widely used for presumptive drug testing, although it does still play a role as a thin layer chromatography stain

15. Denigés' Reagent

The Denigés' reagent was developed in 1898 by G. Denigés is a reagent used for qualitative analysis.

Structure:

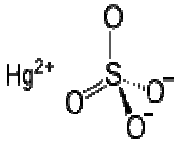

Figure.17 Shows Structure of Deniges reagent

Uses: Denigés' reagent is used to detect is olefin or tertiary alcohols which can be easily dehydrated to form isoolefin in the presence of acid. Treatment of solutions containing either is olefin or tertiary alcohols with this reagent will result in the formation of a solid yellow or red precipitate

Synthesis: Despite the different stoichiometry in these mixtures which varies the concentration of the reagent, they all follow the same idea of adding HgO to distilled water and concentrated sulphuric acid. The Denigés' reagent is ultimately mercury (II) sulphate in an aqueous solution.

- 5 grams of mercury (II) oxide (HgO) is dissolved in 40 mL of distilled water. The mixture is slowly stirred, while 20 mL of concentrated sulphuric acid is added. After adding an additional 40 mL of distilled water, the solution is stirred until the HgO is completely dissolved.
- The Denigés' reagent can also be prepared by dissolving 5 grams of HgO in 20 mL of concentrated sulphuric acid and 100 mL of distilled water.
- The Denigés' reagent can be modified by using nitric acid in place of sulphuric acid

16. Lucas Reagent:

Lucas' reagent is a solution of anhydrous zinc chloride in concentrated hydrochloric acid. This solution is used to classify alcohols of low molecular weight. The reaction is a substitution in which the chloride replaces a hydroxyl group. A positive test is indicated by a change from colourless to turbid, signalling formation of a chloroalkane. The test was reported in 1930 and became a standard method in qualitative organic chemistry. The test has since become somewhat obsolete with the availability of various spectroscopic and chromatographic methods of analysis. It was named after Howard Lucas (1885–1963), an American chemist

Lucas Reagent: Lucas reagent is anhydrous zinc chloride and concentrated hydrochloric acid and is used as a reagent to test alcohols.[18]

Lucas test: Lucas test in alcohols is a test to differentiate between primary, secondary, and tertiary alcohols. It is based on the difference in reactivity of the three classes of alcohols with hydrogen halides .

$$ROH + HCl \rightarrow RCl + H_2O$$

The differing reactivity reflects the differing ease of formation of the corresponding carbocations. Tertiary carbocations are far more stable than secondary carbocations, and primary carbocations are the least stable.

An equimolar mixture of $ZnCl_2$ and HCl is the reagent. The alcohol is protonated by this mixture, and H_2O group attached to carbon is replaced by the nucleophile Cl^-, which is present in excess. Tertiary alcohols react immediately with Lucas reagent as evidenced by turbidity owing to the low solubility of the organic chloride in the aqueous mixture. Secondary alcohols react within five or so minutes (depending on their solubility). Primary alcohols do not react appreciably with Lucas reagent at room temperature. Hence, the time taken for turbidity to appear is a measure of the reactivity of the class of alcohol, and this time difference is used to differentiate between the three classes of alcohols:

- no visible reaction at room temperature and cloudy only on heating: primary, such as normal amyl alcohol (1-Pentanol)
- solution turns cloudy in 3–5 minutes: secondary, such as sec-amyl alcohol (2-Pentanol)
- solution turns cloudy immediately, and/or phases separate: tertiary, such as tert-amyl alcohol (2-Methyl-2-butanol)

Conclusion

In physical and analytical chemistry, **colorimetry** or **colourimetry** is a technique "used to determine the concentration of colored compounds in solution. Colorimetric assays use reagents that undergo a measurable colour change in the presence of the analyte. They are widely used in biochemistry to test for the presence of enzymes, specific compounds, antibodies, hormones and many more analytes.By knowing reagent structure, Principles' and their uses, the drug content from Pharmaceutical dosage forms can be estimated by using spectrophotometrical methods Further investigations on analytical reagents are still going on to find more and more reagents to determine different drug molecules using colorimetry An additional advantage of the spectro photometric methods is that the absorbance is measured at longer wavelengths where the interference from excipients is less and there is no risk of standardisation . From the economical point of view, all the analytical reagents are in expensive, have excellent shelf life and are available in any analytical laboratory. Therefore this method can be recommended for the routine analysis of these drugs in quality control laboratories.

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