1. Introduction

Trimethoprim 2,4-diamino-5-(3,4,5- trimethoxybenzyl) pyrimidine (TMP) is known as folic acid antagonist and is commonly used in combination with sulfanamides to treat gastrointestinal and respiratory tract infections power full bacterioststic agent, (Gangwal and Sharma 1996). Trimethoprim has bacteriostatic effect with broad-range of gram positive and Gram negative bacteria and generally is ineffective to anaerobe, (Barragry 1994). Its main uses now are in Pneumocystis carinii pneumonia, toxoplasmosis, and nocardiosis. Gastrointestinal disturbances (mainly nausea and vomiting) and skin reactions are the most common adverse effects. A high incidence of adverse effects has been reported in AIDS patients; desensitization may sometimes be considered.

1.1 Structure of trimethoprim

Trimethoprim is 5-(3, 4, 5-trimethoxybenzyl) pyrimidin-2,4-diyldiamine.is closed formula $\mathbf{C_{14}H_{18}N_4O_3}$ and molar mass 290.3 g/mol (Figure 1and 2). White and yellowish white colored crystal or crystallized powder. Alternative names Proloprim, Trimpex, Monotrim, Trimexazole and 5-(3,4,5- Trimethoxtbenzyle) , (Yoes,z 1987).

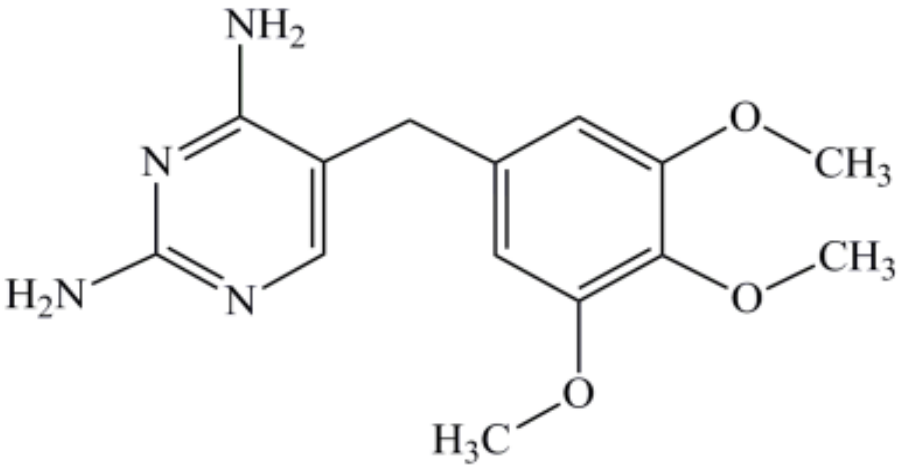


Fig. 1: Chemical structures of trimethoprim

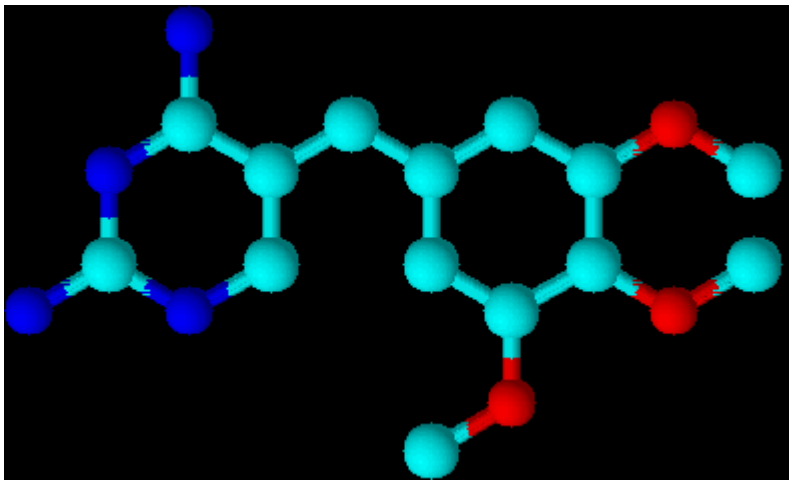


Fig. 2: 3D Chemical structures of trimethoprim

1.2 Physical Description of trimethoprim

Odorless white powder, Bitter taste, white to cream, crystalline powder. (Mcevoy 1992).

1.2.1 Melting Point

199-203 °C 390 to 397 °F

1.2.2 Solubility

Very slightly soluble in water and slightly soluble in alcohol, Soluble in N,N-dimethylacetamide (DMAC) at 13.86; benzyl alcohol at 7.29; propylene glycol at 2.57; chloroform at 1.82; methanol at 1.21; ether at 0.003; benzene at 0.002 g/100 ml at 25 deg C.(Dinç 2011).

1.2.3 Stability

The solubility of trimethoprim in aqueous solution is partially dependent on the pH of the solution, trimethoprim is a weak base and its solubility is lower in solution with a more alkaline pH. Trimethoprim is more stable in injection and stability decrease with increasing concentration.

1.3 Pharmacology and Biochemistry

1.3.1 Pharmacology

Is branch concern with study of drug action where a drug can define as man-made natural endogenous from body. Trimethoprim is a pyrimidine analogue that disrupts folate synthesis, inessential part of the medicine synthesis pathway. Inhibition of the enzyme starves the bacteria of nucleotides necessary for DNA replication. The drug, therefore, exhibits bactericidal activity. (Nadkarni 2005)

1.3.2 Anti malarial agent

Anti malarial agent used in the treatment of malaria, are usually classified on the basis of their action against plasmodia at different stages in their life cycle in the human. Trimethoprim (TMP) is commonly used in combination with sulphamethoxazole (SMZ) as an effective anti-microbial agent. (Lopez 2002).

1.3.3 Mechanism of action of trimethoprim

Trimethoprim is a bacteriostatic lipophilic weak base structurally related to pyrimethamine. Trimethoprim is a synthetic antibiotic that interferes with the production of tetrahydrofolic acid, a chemical that is necessary in order for bacteria and human cells to produce proteins. Trimethoprim inhibits production of tetrahydrofolic acid by inhibiting the enzyme responsible for making tetrahydrofolic acid from dihydrofolic acid. Inhibits the bacterial enzyme more than the corresponding human enzyme. Therefore, TMP has less effect on the production of tetrahydrofolic acid by humans. (Abdulnabi 2008).

.1.4Literature review

Many analytical techniques have been employed for the determination of TMP. The generally used analytical techniques are electrochemical methods (Yaritzky 1991). Some spectrophotometric methods always need previous separation, (Cengic 1986), (British Pharmacopoeia 2002). Other colorimetric methods describe the determination of trimethoprim without prior separation (El-Ansary 1999). Trimethoprim was also analysed in tablet in the presence of sulphadizine using flow injection technique. (Galve 2002). Selective membrane electrode, (Yao 1944). Differential pulsed polarography and cyclic voltammetry,(Petr.Z 2006). TLC (Singletary and Sancilio 1980), A TLC-densitometry, (Madhulika 2011). HPTLC, (KnppI 1986). Ionpair chromatography and spectrodensitomety. (Fazel and Leyla 2006). A biomimetic bulk acoustic wave sensor was fabricated and applied for the

determination of TMP in organic phase based on a molecular imprinting polymer In USP 26; HPLC is used for determination of TMP. However, the determination of TMP and other drugs in biological systems, either individual or combined have been described by HPLC method,(Danijela 2009). Trimethoprim has been determined in pharmaceutical preparations by spectrophotometric methods, (Mahrous 1996). Electro-analytical techniques, (Ahmed,M,A 1995) . Liquid chromatography, (OthmanS 1990). And gas chromatography,(Ernemann 1990). Among others SMZ, on the other hand, has been determined singly using spectrophotometry, (Nagaraja 2002). Fluorimetry (Yarnitzky; 1991). And HPLC ,(Lakkanatinaporn 2004). However, simultaneous determinationof both compounds has been carried out by spectrophotometricmethods with multi-component analysis based on the use of second derivative (Altesor 1993). First derivative and spectral ratio, bivariate calibration spectrophotometrics, (Lopez and Martinez 2002). Other methods include ratio spectra derivative spectrophotometer, (Nevado 1992). Diazotization of SMZ and direct UV measurement of TMP, (Shamsa andAmani 2006). Multivariate calibration approaches (Zhang 2006) and H-point standard additions method, (Givinnrad 2011). The USP (2009) suggests HPLC as the official assay procedure for the quality control of TMP–SMZ combination product while the British Pharmacopoeia (2009) utilizes a sequential method based on extraction by organic solvent .These methods have their peculiar advantages and applicability but many of them are general.

1.5 Pharmaceutical drug analysis

Since the second World War a rapid development of pharmaceutical chemicals , and ultimately drugs , has made a quantum progress . medicinal chemists , pharmacologists , biochemists , analytical chemists and medical professionals have paved the way with their single goal objective to combat the sufferings of human beings . In this integrate

effort the role of an analyst to ascertain the chemical purity of pharmaceutical substances and drugs made their form and finally to determine the dosage forms that are usually available for direct patient's usage , has become not only extremely crucial but also equally important and vital ,as on date product safety has to be an integral part of all product research in pharmaceutical substances . However , the risk –benefit – ratio has got to be pegged to a bare minimum level . Therefore , it has become absolutely necessary to lay emphasis on products Safety research and development which is very crucial in all the developmental stages of a new secondary pharmaceutical product , (Raghad 2006) .

In spite of all the qualified successes of synthetic drug research achieved in the last four decades to combat infectious diseases of the more than 80,000 different ailments , unfortunately only about one third can be treated with drugs , most of them only symptomatically . The discovery of better , effective and safer drugs is needed to fight the causes of dreadful diseases like cancer , acquired – immune – deficiency – syndrome (AIDS) , arthritis , cardio – vascular diseases , disorders of the central nervous system (CNS) , such as Alzheimer's disease and other vital infectious and metabolic diseases like rheumatoid arthritis . In order to meet these challenges on need to adopt novel approaches in pharmaceutical research . Both molecular biology and genetic engineering will be exploited duly in opening up new routes . Genetic engineering may be explored in the development of new drugs , besides , being used as a research to investigate the molecular causes of severe and dreadful diseases .Keeping in view the tremendous global technological competition , one is left with no other choice than to internationalize research and development of pharmaceutical drugs to achieve the common objective (better drugs for a better world) . It is however , pertinent to mention here that pharmaceutical chemicals must maintain a very high degree of

chemical purity .It is quite obvious that a state of absolute purity may not be achievable , but a sincere effort must be exercised to obtain the maximum freedom from foreign substances . Bearing in mind the exorbitant operational costs to attain the (highest standards) of purity , perhaps some of these processes are not economically viable . Therefore , a compromise has got to be made to strike a balance between the purity of a substance at a reasonably viable cost and at the same time its purity e.g., being fully acceptable for all pharmaceutical usages . In short , a host of impurities in pharmaceutical chemicals do occur that may be partially responsible for toxicity chemical interference and general instability. (Raghad 2006).

1.6 Techniques of pharmaceutical analysis

The advanced spectrometric methods of analysis including UV – Visible IR , MS , NMR techniques in addition to phosphorescence spectrometry flame emission and atomic absorption spectroscopy . Chromatographic methods are also included with special emphasis on the coupled techniques of GC / MS & LC / MS , (Raghad 2006).

1.6.1 Spectrophotometric methods

Spectrophotometer is an instrument which is capable of isolating (monochromatic) radiation, or that which specifically contains a dispersing element ,a prism or a grating . It is pertinent to mention here that there are a plethora of commercially available spectrophotometers of varying design i.e., single – beam (simple) , double - beam (more precise and accurate) and microcomputer controlled built – in – recorder with separate printer ; and obviously having a wide – price – range form . Evidently , it is practically impossible to describe either all or even a major fraction of , the various spectrophotometers available . Therefore , in this particular section the following two types of spectrophotometers shall be discussed briefly, (Raghad 2006) .

(a) Single – beam spectrophotometer.

(b) Double – beam spectrophotometer.

1.6.1.1 Single beam spectrophotometer

The desired wavelength is isolated by using a prism or grating and auxiliary mirrors and slits that collectively form a microchromator of the instrument . The wavelength dial on a spectrophotometer is adjusted to a specific value , but the radiation leaving the exit – slit is found to be rarely monochromatic (Raghad 2006) .

1.6.1.2 Double beam Spectrophotometer

The quantum leap amalgamated with qualified success in the advancement of analytical instruments necessitated for more rapid , precise and accurate measurements in UV and visible spectroscopy .It could be accomplished by the help of the following two cardinal modifications , namely :

(a) Need for a continuous change in wavelength so that light through the blank and through the sample may be monitored continuously .

(b) Measurements done with a recording spectrophotometer. The above two modifications have been duly in a double – beam spectrophotometer .

IR –Spectroscopy in the analysis of pharmaceutical , a host of pharmaceutical substance can be identified and critically examined with the help of infrared spectroscopy . Hence , the latest versions of British pharmacopoeia (BP) and United States pharmacopoeia (USP) contain the compendium . The complete IR – spectrum of such pure pharmaceutical substances that are essentially included in the respective official compendium .These authentic IR – spectra are profusely used in many well equipped Quality Assurance Laboratories in checking the purity of commercially available drugs before employing them in various formulations . Following is the detailed procedure laid out in the

pharmacopoeia of India (IP) for the preparation of KBr – disc or KCl –disc , (Raghad 2006) .

1.6.2 Chromatographic method

Chromatographic method is considered one of the most important methods used to determine trimethoprim . Many research published work for pharmaceutical tablet or pure form. Determined trimethoprim in plasma sample using GAS chotomatography with helium as mobile phase, and also he used GC/MS (Hasegawa 1995). Trimethoprim determined by using Thin-layer choromarogrphy (TLC), (Zhang 2000). Deshapande used TLC and flame ionisation by 1% acetic acid /methanol as mobile phase. And also by TLC π-acceptors as detector for example 2.5-dichloro-p-benzoquinone in DMSO solution.The DMSO was dissolved in aceto or methanol and separated by TLC using ethyl acetate / methanol 9:1 as mobile phase after that its dried to give clear color (Agarwal 1990). High performance Tine-Layer chromatography (HPTLC) by dissolve TMP in ethanol / chromphorm as mobile phase (Lalla 1997). Another chromatography method to determined trimethoprem it is Micellar Electro kinetic capillary chromatography (MEKCC) and also by capillary Zone Electro phpresis (CZE), (Lemus 1988-1991).

1.6.3 Titrimeric methods

TMP was determined by titrimeric method by using 0.2g of pharmaceutical tables in hot glacial acetic acid the solution was filtered, washed by distilled water, crystal violet in detector add and titrated against $HCLO_4$ the blue colour end point ,**(**Yue 1993).

1.7 Charge – Transfer complexes

A charge – transfer complex (CT complex) or electron donor-acceptor complex is a chemical association of two or more molecules, or of different

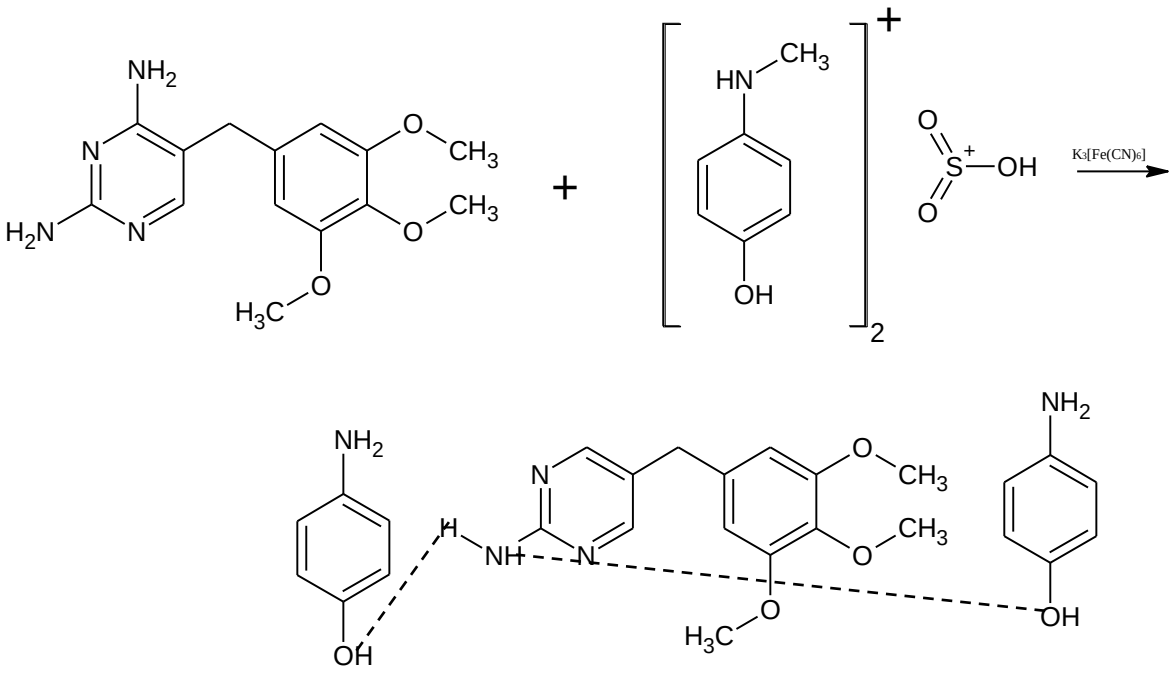
parts of one very large molecule, in which the attraction between the molecules (or parts) is created by an electronic transition into an excited state, such that a fraction of electronic charge is transferred between the molecular entities. The resulting electrostatic attraction provides a stabilizing force for the molecular complex. The source molecule from which the charge is transferred is called the electron donor and the receiving molecule is called the electron acceptor. The nature of the attraction in a charge-transfer complex is not a stable chemical bond and is much weaker than covalent forces; rather it is better characterized as a weak electron resonance. As a result, the excitation energy of this resonance occurs very frequently in the visible region of the electro-magnetic spectrum. This produces the usually intense colors characteristic for these complexes. These optical absorption bands are often referred to as charge-transfer bands (CT bands). Optical spectroscopy is a powerful technique to characterize charge-transfer bands.

Charge – transfer complexes exist in many types of molecules, inorganic as well as organic, and in all phases of matter, i.e. in solids, liquids, and even gases. That to say charge – transfer complexes were formed by the interaction between electron donors and electron acceptor using valence bond theory (*Coats A. W and Redfern 1954).* In this theory one or more electron were transferred from the donors to the acceptors component of the complexes. The observed electronic absorption spectra, characteristic of the complex as a whole, were accountable as intermolecular charge transition (Charge-transfer). The donors may be n- or π type e.g. polycyclic aromatic hydrocarbons are π- type, and those containing lone pair of electrons are n- donors. Some compounds such as azo aromatic and amines, may be have as n- donors to some acceptors and π- donors towards other acceptors may be π - or π- type. The π - acceptors may be aromatic systems containing electron-withdrawing substituents such as nitro, cyano, or halogen groups, acid chloride, anhydrides and quinines belonging to the same class of acceptors.

A charge- transfer method was utilized for spectrophotometric determination of trimethoprem drug, which depends on reaction of metol and potassium hexacyanoferrate (III) in aqueous media to form CT-complex. Trimethoprem contains for nitrogen atom acts as n- electron donor to metol which acts as π-acceptor in potassium hexacyanoferrate(III) as intermediate giving brown colored complex. Influence of different parameters, such as time, temperature, and pH and stoichometric ratio was studied spectrophotometrically to determine the optimum conditions for the proposed method.

1.8 The objectives of the research work

This research show the reaction between the drug trimethoprem and metol potassium hexacyanoferrate (III) is affected by various variables. The aim of this research work to establish the optimum conditions by testing the variables like effect of: pH, volum of metol and potassium hexacyanoferrate (III) , volum of TMP , temperature and time . This research aimed also to shed light on the reactions of metol and potassium hexacyanoferrate (III) trimethoprim drug in solutions. These reactions are take place through charge transfer with hydrogen proton in metol, and nitrogen group in trimethoprim drug.



Charge transfer complexes

Fig ١.3: Reaction scheme

2. Materials and methods

2.1 Materials and reagents

All reagents used were of analytical grade and water was always distilled and of highest purity degree available, and authentic samples were kindly supplied by AMEFARMA pharmaceutical company , Khartoum North _ Sudan . Trimethoprim tablets labelled to contain (80mg). They were obtained local market pharmacy. The melting points of the marked drug was determined at 237 C° . And also the melting points of metol reagent were determined at 255 C°. Trimethoprim (TMP, M.Wt = 290.3 g/mol) potassium hexacyanoferrate (III) $K_3[Fe(CN)_6]$ (M.Wt= 329.24 g/mol). And organic reagent metol M.wt(344.4g/mol). And also methanol (CH_3OH, M. Wt = 32 g/mole). Sodium hydroxide (NaOH , M. Wt = 40 g/mole), hydrochloric acid (HCl, M. Wt =36.5 g/mole), phosphoric acid (H_3PO_4, M. Wt 98 g/mole), acetic acid (CH_3COOH, M. Wt =60 g/mole) . Boric acid (H_3BO_4, M. Wt =77.8 g/mol). Pharmaceutical preparations of the investigated drugs were purchased from local markets.

2.2 Preparations of solutions

2.2.1 Stock solution of trimethoprim

Fresh stock solution of (0.01 M) (290,32 mg. ml^{-1}) of TMP drug was prepared by dissolving the accurately weighed 2.9g and transferred into 50 ml beaker .A mixture consisted of 10-ml methanol and 10-ml distilled water (1 : 1) were used to dissolved TMP . The solution was transferred into 1000 -ml volumetric flask and completed to the mark by using distilled water.

2.2.2 Stock solution of metol

Fresh stock solution of (0.01M) metol was prepared by dissolving the accurately weighed (0.68889g) by 5-ml distilled water and diluting to 100-ml with distilled water in volumetric flask.

2.2.3 Stock solution of Potassium hexacyanoferrate (III)

Fresh stock solution of (0.01 M) Potassium hexacyanoferrate (III) was prepared by dissolving (0.6888 g) by 5-ml distilled water and complete to 100-ml with distilled water in volumetric flask.

2.2.4 Preparation of Buffer solution

Series of universal buffer solutions covering the range of pH values from 2.0 to 11.0 were prepared as recommended by Britton and Robinson (Britton H. T. S 1990). A mixture of 0.04 M phosphoric, acetic and boric acids was titrated with 0.2 M NaOH to adjust the desired pH into the required value in 100 ml of the acid mixture using pH –meter. The (0.01M) disodium salt of EDTA solution (M. wt = 372 g/ mol) was prepared by dissolving 0.93 g in 250-mL. This solution was standardized, (Khalifa ,H 1996).

2.2.5 Stock solutions of pharmaceutical preparations

Each ten tablets of TMP (80 mg/tablets) were weighed and powdered well separately . Equivalent amount of powder to one tablets of trimethoprim drug was weighed and dissolved in mixture of methanol and water (1:1) . The solution of drug was transferred into separate 50 - ml volumetric flask and the volume completed to the mark with distilled water .The resulting solution was shake well and kept .

2.3 Instruments

Automated Spectrophotometer Spectronic model T80 UV / Vis spectrometer PG Instruments Itd , with matched quartz cell of 1 cm optical length, ranged from 200 - 1000 nm. pH measurements were performed by using Hanna PH211 meter: Inc Woonsocket – RI – UA made in Romania. Also, sensitive analytical balance: 0.0001g, SCALTEC (Germany) was used for weighing. Moreover, magnetic stirrer with thermostatic hot plate (VELP-Europe) was used for stirring and heating of solution, pipettes Accupipettes, USA (10- 100 , 100-1000) ml.

2.4 Methods

2.4.1 Preparation of complex compound

Solutions of standard TMP (0.01M) was diluting to (0.001M) and (0.0001M) respectively. And also solutions of metol and potassium hexacyanoferrate (III) were diluting (0.001M) and (0.0001M) respectively, mixture of product were prepared by mix of TMP with metol and potassium hexacyanoferrate (III) in different volume to obtain suitable wavelength.

2.4.2 Spectrophotometric determination of standard trimethoprim drug

2.4.2.1 Selection of the wavelengths

I: Solution of (0.001M) of TMP was scanned in UV / Vis spectrometer at the wavelength range 200 to 800 nm, in order to determine the λ_{max} of the standard drug to select suitable wavelength.

II: Equal volume of mixture of reagent potassium hexacyanoferrate (III) and metol were scanned in the wavelength range 200 to 800 nm, in order to determine the λ_{max} of metol).

III: mixture of TMP and reagent were scanned in the wavelength range 400 to 800 nm.

2.4.2.2 Effect of time and temperature on the spectra of drug reaction products

Effect of time and temperature ranged from (0 to 50 min) and (25 to 65 °C), respectively, were studied on the spectra of drug reaction products with potassium hexacyanoferrate (III) and metol) reagents. The spectrophotometric measurements were recorded at 420 nm for TMP reaction products in table: 3.1, 3.2 respectively.

2.4.2.3 Effect of pH on product

Series of universal buffer solutions were prepared in 10 volumetric flask and added equal volume of mixture of TMP and product effect of pH for TMP was measured in 1-12 range of pH the buffer solution was add to give the required pH, the measurements were recorded at 420 nm for TMP reaction product.

2.4.2.4 Effect of volume of potassium hexacyanoferrate (III)

The effect of the different volumes of k_3 [Fe $(CN)_6$] solution was examined on the maximum absorbance of the colored product in the presence of (1-ml)(2-ml) from mixture of metol and potassium hexacyanoferrate (III) respectively.

2.4.2.5 Stoichiometric ratio

The molar ratio method (MRM) (Yoe J.H, and Jones 1944) was applied to determine the suitable stoichiometric ratio (drug: reagent, D: R). A series of drug solutions for TMP was prepared in which the reagent concentration was kept constant (0.001M) to which variable concentrations of drug solution (0.001- 0.0001 M) were added. The spectrophotometric measurements of these solutions were recorded at the suitable maximum wavelengths (λ_{max}) 420 nm for trimethoprim reagent product with marked reagent .

2.4.2.6 Validity of Beer's Law

Under the optimum conditions describe above (λ_{max} , time , temp pH , volume of metol , k_3 [Fe $(CN)_6$] and stoichiometric ratio) the calibration curves of TMP was constructed . Linearity of Beer's law was determined for drug reagent product. A low and high detection limit of calibration curves was determined.

2.4.3 Pharmaceutical application

Solutions of pharmaceutical preparations were prepared as given under solutions of pharmaceutical preparations for the proposed method was applied to determination of trimethoprim in tablets by the analysis of three concentrations of sample using the recommended procedure.