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FORMULATION AND EVALUATION OF ANTIMALARIAL DRUGS SUPPOSITORIES

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ABSTRACT

Great progress has been made in recent years to reduce the high level of suffering caused by malaria worldwide. Notably, the use of insecticide-treated mosquito nets for malaria prevention and the use of artemisinin-based combination therapy (ACT) for malaria treatment have made a significant impact. Nevertheless, the development of resistance to the past and present anti-malarial drugs highlights the need for continued research to stay one-step ahead. The present study was aimed to formulate, evaluate and optimized of antimalarial drugs as suppository dosage forms different formula of Chloroquine phosphate, Pyrimethamine, Sulfadoxine a combination antimalarial containing the sulfonamide antibacterial Sulfadoxine and the antiparasitic Pyrimethamine, it is used to prevent and treat malaria. The rectal drug delivery can be alternative to oral route and convenient for infants, children and the elderly who find it difficult to swallow tablets. Alternative methods of Chloroquine phosphate administration such as rectal delivery to avoid the bitter taste and improve patient comfort and compliance. In the present study, attempt has been made to prepare drugs as suppository dosage forms of Chloroquine phosphate, Pyrimethamine, Sulfadoxine using suppository bases like Cocoa

butter, Polyethylene glycol (PEG4000) and Witepsol H35 using different concentration of suppository bases the parameters of prepared suppository dosage forms melting point, weight variation, disintegration time, invitro dissolution release rate were evaluated. The present study found that among the all formulations F3(IV) Chloroquine phosphate formulation was found to be showed improved drug release characteristics. F3(IV), which consists of (Chloroquine phosphate and PEG4000 base), showed the best result in drug release for antimalarial drugs as suppository dosage forms.

KEYWORDS: Suppositories, Chloroquine phosphate, Pyrimethamine, Sulfadoxine, Malaria.

INTRODUCTION

Malaria is an infectious disease caused in human by the *Plasmodium spp*., which infects erythrocytes. There are over one hundred species of the genus *Plasmodium*; however, only five of these have been shown to infect humans, viz. *P. vivax*, *P. ovale*, *P. knowlesi*, *P. malariae*, and the most prevalent, *P. falciparum*.[1-3]

Recently *P. knowlesi*, has been causing tertian malaria in Malaysia and areas of Southeast Asia. Another species, *P.falciparum*, affects more erythrocytes than the other species and is much more serious and fatal within a few hours of the first symptoms.[4-9]

Malaria remains a global public health problem affecting nearly half of the world's population. In the year 2020, global estimates indicated 241 million malaria cases and 627000-malaria deaths.[1] In Yemen, 99% recorded cases had malignant malaria and annual incidence was not less than one million cases.[3] Most people diagnosed in the U.S. obtained their infection outside of the country, usually while living or traveling through an area where malaria is endemic.[4-6]

Chloroquine has been the drug of choice for protecting malaria infections. However, because of resistance, it is now only suggested for use in areas where *P. vivax*, *P. oval*, and *P. malaria* are present. *P.falciparum* that caused malignant malaria is becoming increasingly resistant to anti-malarial medications. For travelers going to areas where *P.falciparum* is endemic like in Yemen, there are several options for malaria prevention, including mefloquine, atovaquone/proguanil (Malarone), and doxycycline.[4-8]

The most effective treatment for *P. falciparum* infection is the use of artemisinins in combination with other antimalarials (known as artemisinin-combination therapy, or ACT),

which decreases resistance to any single drug component. These additional antimalarials include: amodiaquine, lumefantrine, mefloquine or Sulfadoxine /Pyrimethamine.[10-15]

For severe malaria that caused by *P.falciparum*, artesunate is superior to quinine in both children and adults.[4-11] In many parts of the world, for instance, resistance to Chloroquine has rendered the drug ineffective.

The suppository may be ideally used in: Babies or old people who cannot swallow oral medication and drugs destroyed by portal circulation.

The quantity of fluid available for drug dissolution is very small (approximately 3 ml). The rectal fluid is neutral in pH (7-8) and has no buffer capacity and when systemic effects are desired, greater absorption may be expected from an empty rectum as the drug will be in good contact with the absorbing surface of the rectum. Thus, the dissolution of slightly soluble substances is the slowest step in the absorptive process.[11-27]

Intra-rectal administration represents a promising administration route of anti -malarial medicines in the field, and is especially convenient for patients un-able to swallow and when parenteral formulations are unavailable or cannot be administered. Results from a variety of clinical studies have indicated that artemisinin- based suppositories can be used for initial emergency and curative treatment in un complicated, moderate, severe and cerebral malaria.[2]

Chloroquine phosphate

A white or almost white, crystalline powder, hygroscopic, freely soluble in water, very slightly soluble in alcohol and in methanol. It is in a class of drugs called antimalarials and amebicides. It is used to prevent and treat malaria. It is also used to treat amebiasis and it may cause, headache, loss of appetite, diarrhea, upset stomach, stomach pain, skin rash or itching, hair loss, mood or mental changes.[11,12]

Sulfadoxine

White or yellowish-white crystalline powder or crystals, very slightly soluble in water, slightly soluble in alcohol and in methanol. It dissolves in solutions of alkali hydroxides and in dilute mineral acids. It melts at about 99°C, with decomposition. Store protected from light. It is used in combination with pyrimethamine to treat or prevent malaria. It is also used, usually in combination with other drugs, to treat or prevent various infections in livestock.[15]

Pyrimethamine

Almost white, crystalline powder or colorless crystals, practically insoluble in water, slightly soluble in alcohol. Store protected from light. It is used for protozoal infections. It is commonly used as an antimalarial drug (for both treatment and prevention of malaria) and is also used (combined with sulfadiazine) in the treatment of *T. gondii* infections in immunocompromised patients.[12-18]

Sulfadoxine/ Pyrimethamine (Combination)

A combination antimalarial containing the sulfonamide antibacterial Sulfadoxine and the antiparasitic pyrimethamine Both drugs are anti folates; they inhibit the production of enzymes involved in the synthesis of folic acid within the parasites. *P. falciparum* may be able to use exogenous folic acid, i.e. folic acid which is present in the parasite's environment, while in combination, the two substances have a synergistic effect which outbalances that ability. The combination is considered to be more effective in treating malaria caused by *P. falciparum* than that caused by *P. vivax*. Due to side effects, however, it is no longer recommended as a routine preventative, but only to treat serious malaria infections or to prevent them in areas where other drugs may not work.[10-20]

The present study was aimed to formulate and evaluate an antimalarial drugs using different suppository bases and select the best one.

MATERIALS AND METHODS

Pyrimethamine (R.L. flne chem.), Sulfadoxine (Pharma chem. Pharmaceutical), Chloroquine phosphate (IPCA laboratories limited India) all as a gift from (Shaphaco Pharmaceutical Company-Yemen), Cocoa Butter (M.O.H Kuwait), Witepsol H35 (Sasol gulf), Liquid paraffin, PEG 4000 as a gift from (Pharmacare Pharmaceutical Industry Company-Yemen).

Equipment's: Melting point (Stuart scientific, U.K), I.R (FT/IR Spector, FT.IR), Heater (Assisstent, Schott, Germany), Disintegration Tester (Pharma-max-Test, d-63512, Germany), Dissolution Tester (PTW5610, Germany), HPLC (Water 1525 binary HPLC pump including online degasser, Germany), Mold: (Germany capacity : 2gm), Magnetic starrier (Germany), Balance (Germany, U.V (UV/VIS Spectrophotometer (Model : 50 Conc, Germany).

Preformulation Studies

Melting Point Determination

by using melting point apparatus according to the British Pharmacopeia.

IR Spectroscopy Test

An interferogram of sample was used to obtain the spectrum of the sample. After an interferogram has been collected, a computer performs a Fast Fourier Transform, which results in a frequency domain trace that we all know and love. For a chive a good signal to noise ratio, many interferograms are obtained and then averaged. This can be done in less time than it would take a dispersive instrument to record one scan.

Displacement Value D.V Determination

The displacement value may define as the number of part by weight of medication that displaces one part by weight of the base. The displacement value for Pyrimethamine, Chloroquine phosphate and Sulfadoxine and combination we are calculated using these lows to calculate of D.V and the values were within the normal acceptable range and were taken into consideration in the formation.

Preparation of Suppository Different Bases

The total quantity of base required to fill the nominal capacity (2gm), mold lubrication with liquid paraffin using quiz and any excess lubrication drain. The weighted base about (x gm), heated to melt then the molten mass poured cavity mold and cool then the excess was removed.

Preparation of Active Ingredients Suppositories Using Different Bases

The quantity required of Cocoa butter, PEG4000, Witepsol H35 and Pyrimethamine Sulfadoxine combination and Chloroquine phosphate were calculated as illustrated in Table 1.

Weight the suppositories and D.V values were calculate as shown in Tables 2 to 5.

Suppository Formulations

The quantities were calculated as shown in Table 5 to 9. Where weight of drug = concentration of drug x no. of suppositories x capacity of mold. Amount of base displace by drug = weight of drug / D.V While the amount of base for drug (required) = no. of suppositories –amount of base. Percent of error = weight of drug / no. of suppositories.

Table 1: Preparation of Suppository Formulations of Pyrimethamine, Sulfadoxine, Chloroquine Phosphate and Pyrimethamine / Sulfadoxine (combination).

Bases	Pyrimethamine (I)	Sulfadoxine (II)	Combination (III)	Chloroquine.P. (IV)
(F1) Cocoa B	Rx Pyrimeth. 0.4gm CocoaB 15.62gm	Rx Sulfadoxine8gm CocoaB 7.895gm	------	Rx Chloroq.P.4.8gm CocoaB.11.23gm
(F2) Witepsol H35	Rx Pyrimeth.0.4gm Witepsol15.808gm	Rx Sulfadoxine 8gm Witepsol 8.2gm	------	Rx Chloroq.P4.8gm Witepsol11.25gm
(F3) PEG4000	Rx Pyrimeth. 0.4gm PEG 15.68gm	Rx Sulfadoxine 8gm PEG7.6 gm	Rx Pyrimeth0.4gm Sulfadoxine8gm PEG 16gm	Rx Chloroq. P4gm PEG 11.3gm
(F4) Cocoa Butter & Witepsol H35	Rx Pyrimeth. 0.4gm CocoaB.7.801gm Witepsol7.005gm	Rx Sulfadoxine 8gm CocoaB.4.01gm Witepsol 4.01gm	Rx Pyrimeth.0.4gm Sulfadoxine8gm CocoaB 4.01gm Witepsol4.01mg	Rx Chloroq.P.4.8gm CocoaB.5.6gm Witepsol5.605gm
(F5) Cocoa Butter & PEG4000	Rx Pyrimeth. 0.4gm CocoaB.7.801gm Witepsol7.01gm	Rx Sulfadoxine.8gm CocoaB4.05gm PEG4.05 gm	-------	Rx Chloroq.P.4.8gm CocoB5.62gm PEG 5.62gm
(F6) Witepsol H35 & PEG4000	Rx Pyrimeth. 0.4gm Witepsol7.86gm PEG7.86gm	Rx Sulfadoxin 8gm Witepsol 3.8gm PEG 3.8gm	--------	Rx Chloroq.P.4.8gm Witepsol 5.63gm PEG5.63gm

Table 2: D.V Determination of Pyrimethamine Suppositories.

Bases	Cocoa Butter	Witepsol H35	PEG 4000	Cocoa Butter + Witepsol H35	Cocoa Butter + PEG4000	PEG4000 + Witepsol H35
Wt. of Base	16 gm	16 gm	16 gm	16 gm	16gm	16 gm
Wt. of Drug (D)	0.4 gm	0.4 gm	0.4 gm	0.4 gm	0.4gm	0.4 gm
Withdraw Sample	1.8 ml	2 ml	2 ml	2 ml	2 ml	2 ml
Wt. of Supp. with Med. (A)	12.64 gm	12.632 gm	15.25 gm	11.83 gm	13.26 gm	13.65 gm
Wt. of Supp. without Med. (C)	12.00 gm	12.60 gm	15.20 gm	11.76 gm	13.20 gm	13.60 gm
(A -C)	o.64 gm	0.03gm	0.05 gm	0.067 gm	0.058 gm	0.047gm
D.V = D/B(A-C)	0.4/6(0.64) = 1.04	0.4/6(0.032) = 2.08	0.4/6(0.05) = 1.25	0.4/6(0.07) = 1.003	0.4/6(0.06) = 1.143	0.4/6(0.05) = 1.43

Table 3: D.V Determination of Sulfadoxine Suppositories.

Bases	Cocoa Butter	Witepsol H35	PEG 4000	Cocoa Butter + Witepsol H35	Cocoa Butter + PEG4000	PEG4000 + Witepsol H35
Wt. of Base	16 gm	16 gm	16 gm	16 gm	16 gm	16 gm
Wt. of Drug (D)	8gm	8gm	8gm	8gm	8gm	8gm
Temp.	66 °C	66 °C	66 °C	66 °C	66 °C	66 °C
Withdraw Sample	1.9 ml	1.8 ml	2 ml	2 ml	2 ml	2 ml
Wt. of Supp. with Med. (A)	13.90 gm	13.35 gm	16.60 gm	13.09 gm	14.52 gm	14.55 gm
Wt. of Supp. without Med. (C)	12 gm	12.6 gm	15.2 gm	11.76 gm	13.20 gm	13.60 gm
(A -C)	1.35 gm	1.3 gm	1.4 gm	1.33 gm	1.3 gm	0.16 gm
D.V = D/B(A-C)	8/6(1.35) = 0.987	8/6(1.30) = 1.026	8/6(1.40) = 0.952	8/6(1.33) = 1.003	8/6(1.32) = 1.012	8/6(0.16) = 1.400

Table 4: D.V Determination of Pyrimethamine and Sulfadoxine (Combination) Suppositories.

Bases	PEG4000	Cocoa Butter+ Witepsol H35
Wt. of Base	16gm	CocoaB 8 gm + Witepsol H35 8gm =16gm
Wt. of Drug (D)	Sulfadoxine 8gm + Pyrimethamine0.4gm =8.4gm	Sulfadoxine 8gm +Pyrimethamine 0.4gm =8.4gm
Temp.	60C°	60C°
Time	3h.	3h.
Withdraw Sample	2ml	2ml
Wt. of Supp. with Med. (A)	16.6gm	13.09gm
Wt. of Supp. without Med. (C)	15.2gm	11.76gm
(A -C)	1.4gm	1.33gm
D.V = D/B(A-C)	8.4/6(1.33) = 0.952	8.4/6(1.33) = 1.052

Table 5: D.V Determination of Chloroquine Phosphate Suppositories.

Bases	Cocoa Butter	Witepsol H35	PEG 4000	Cocoa Butter+ Witepsol H35	Cocoa Butter+ PEG4000	PEG4000 + Witepsol H35
Wt. of Base	16 g	16 g	16 g	16 g	16 g	16 g
Wt. of Drug (D)	4.8 g	4.8 g	4.8 g	4.8 g	4.8 g	4.8 g
Temp.	100 °C	100 °C	100 °C	100 °C	100 °C	100 °C
Time	3 h	3 h	3 h	3 h	3 h	3 h
Withdraw	1.9 ml	2 ml	2 ml	2 ml	2 ml	2 ml

Sample						
Wt. of Supp. with Med. (A)	12.80 gm	13.39 gm	15.98 gm	12.56 gm	13.99 gm	4.39 gm
Wt. of Supp. without Med. (C)	12gm	12.60 gm	15.20 gm	11.76 gm	13.20 gm	13.60 gm
(A -C)	0.795 gm	0.792 gm	0.784 gm	0.798 gm	0.793 gm	0.790 gm
D.V = D/B(A-C)	4.8/6(0.795) = 1.006	4.8/6(0.792) = 1.010	4.8/6(0.784) = 1.021	4.8/6(0.798) = 1.002	4.8/6(0.793) = 1.0084	4.8/6(0.79) = 1.01265

Table 6: Calculation of The Suppositories of Pyrimethamine prepared Formulations (Each Suppository Contain Pyrimethamine 50mg).

Bases	Cocoa Butter	Witepsol H35	PEG 4000	Cocoa Butter+ Witepsol H35	Cocoa Butter+ PEG4000	PEG4000+ Witepsol H35
Wt. of Drug = Conc. x No of Supp. x Capacity of Mold	0.025x8x2 = 0.4	0.025x8x2 = 0.4	0.025x8x2 = 0.4	0.025x8x2 = 0.4	0.025x8x2 = 0.4	0.025x8x2 = 0.4
Amount of Base = Wt. of Drug / D.V.	0.4/1.428 =0.28	0.4/1.1428 =0.35	0.4/1.0025 =0.399	0.4/1.25 =0.32	0.4/2.0833 =0.192	0.4/1.04 =0.38
Amount of Base for Drug.	8-0.28 =7.72	8-0.35 =7.65	8-0.399 =7.601	8-0.32 =7.68	8-0.192 =7.807	8-0.38 =7.62
Theoretical Correction for D.V.	7.72+0.4 =8.12	7.65+0.4 =8.05	7.601+0.4 =8.001	7.68+0.4 =8.08	7.807+0.4 =8.20	7.62+0.4 =8.02

Table 7: Calculation of The Suppositories of Sulfadoxine Prepared Formulations (Each Suppository Contain Sulfadoxine1000 mg).

Bases	Cocoa Butter	Witepsol H35	PEG 4000	Cocoa Butter+ Witepsol H35	Cocoa Butter+ PEG4000	PEG4000+ Witepsol H35
Wt. of Drug = Conc. x No of Supp. x Capacity of Mold	0.5x8x2 = 8	0.5x8x2 = 8	0.5x8x2 = 8	0.5x8x2 = 8	0.5x8x2 = 8	0.5x8x2 = 8
Amount of Base = Wt. of Drug / D.V.	8/0.952 = 8.4	8/1.012 = 7.9	8/1.0025 =7.98	8/0.952 = 8.4	8/1.0256 = 7.80	8/0.987 = 8.11
Amount of Base for Drug.	8-8.4 = 0.4	8-7.9 = 0.1	8-7.98 = 0.02	8-8.4 = 0.4	8-7.80031 = 0.2	8-8.1053 = 0.105
Theoretical Correction for D.V.	8+0.4 = 8.4	8+0.1 = 8.1	8+0.02 = 8.02	8+0.4 = 8.4	8+0.2 = 8.2	8+0.1053 = 8.1

Table 8: Calculation of The Suppositories of Pyrimethamine nda Sulphadoxine Combination Formulations (Each Suppository Contain 50mg Pyrimethamine and 1000 mg of Sulphadoxine).

Bases	Cocoa Butter and Witepsol H35	4000PEG
Wt. of Drug = Conc. x No of Supp. x Capacity of Mold	0.5x8x2=8 0.025x2x8=0.4 0.4+8=8.4	0.5x8x2=8 0.025x2x8=0.4 0.4+8=8.4
Amount of Base = Wt. of Drug / D.V.	8.4/0.952= 8.823gm	8.4/1.0526= 7.98
Amount of Base for Drug.	8-8.823=0.823gm	8-7.98=0.02gm
Theoretical Correction for D.V.	8+0.823=8.823gm	8+ 0.02 =8.02

Table 9: Calculation of The Suppositories of Chloroquine Phosphate Formulations (Each Suppository Contain 600 mg Chloroquine Phosphate).

Bases	Cocoa Butter	Witepsol H35	PEG 4000	Cocoa Butter+ Witepsol H35	Cocoa Butter+ PEG4000	PEG+ Witepsol H35
Wt. of Drug = Conc. x No of Supp. x Capacity of Mold	0.3x8x2 = 4.8	0.3x8x2 = 4.8	0.3x8x2 = 4.8	0.3x8x2 = 4.8	0.3x8x2 = 4.8	0.3x8x2 = 4.8
Amount of Base = Wt. of Drug / D.V.	4.8/1.006 = 4.77	4.8/1.01265 = 4.74	4.8/1.0084 = 4.76	4.8/1.002 = 4.79	4.8/1.021 = 4.7	4.8/1.010 = 4.75
Amount of Base for Drug.	8-4.77 = 3.23	8-4.74 = 3.26	8-4.76 = 3.24	8-4.79 = 3.21	8-4.7 = 3.3	8-4.75 = 3.25
Theoretical Correction for D.V.	4.8+3.23 = 8.03	4.8+3.26 = 8.06	4.8+3.24 = 8.04	4.8+3.21 = 8.01	4.8+3.3 = 8.05	4.8+3.25 = 8.05

Evaluation of Suppositories[28]

As shown in Table 10.

Weight Variation

Twenty suppositories taken random. And weighted individually, NMT2 of individual weight deviate from the average weight by more than ±5% deviation and non-deviates by more than twice that percentage.

Disintegration Test

Suppositories were weighed, placed in disintegration tester and the state of the sample examine after 30 minutes for fat-based suppositories, and after 60 minutes for water-soluble based suppositories.

Dissolution Test

Dissolution for Pyrimethamine, Sulfadoxine and Combination (Pyrimethamine with Sulfadoxine)

Using apparatus II (basket), medium of 1000 ml 0.05M phosphate buffer, pH 7.8 rotate at 75 rpm for 30 minutes.

Medium preparation: Weigh 47.64 gm of Potassium dihydrogen phosphate dilute to 7000ml add 1.5gm of potassium hydroxide pellets (adjust pH to 7.8 + 0.1 if necessary by 1M KOH).

Standard preparation: Prepared as directed in the assay method.

Procedure: One suppository was placed in each dissolution vessel and operate the apparatus at 75 rpm for 30 minutes. Determine the amount of active ingredients dissolved in the dissolution medium as directed in the assay method, using instead of sample solution 10 ml a filtered portion of the solution under test diluted to 20 ml by mobile phase.

$$\text{Dissolved amount} = \frac{\text{Area of sample} \times \text{Conc. of std.}}{\text{Area of standard} \times \text{Conc. of sample}} \times \text{purity of std.}$$

Dissolution Test of Chloroquine Phosphate

using as the medium 900 ml of 0.1M hydrochloric acid and rotating the basket at 100 revolutions per minute. Withdraw a sample of 10 ml of the medium. Measure the absorbance of a layer of suitable thickness of the filtered sample, suitably diluted if necessary, at the maximum at 344nm. Calculate the total content of Chloroquine phosphate, C18H26ClN3, 2H3PO4, in the medium taking 371 as the value of A (1%, 1 cm) at the maximum at 344 nm. medium 0.1 N HCl 900ml time 30minutes, Rotation75 rpm, Wave length 344nm.

Preparation of standard solution: take eq. wt 66mg from Chloroquine phosphate in to 100ml of volume flask 2- dissolve by 0.1 N HCl take 2ml in to 100ml volume flask and complete the volume by media The concentration = 0.0132 mg /ml reading at 344nm.

Preparation of sample solution

At the end of dissolution time (30 minutes) from the filtrate, 2ml was taken into 100ml volume flask and complete the volume by 0.1N HCl.

Assay of Sulfadoxine

Each suppository contains 1000 mg Sulfadoxine. HPLC conditions where: Column: C18 5µm 4.6 x 150 mm, Flow rate: 2.0 ml / min., Wavelength: 240nm. Mobile phase: Prepare degassed and filtered mixture of 800ml of 0.01M phosphoric acid and 200ml Acetonitrile, filter by

0.45um Nylon micromembrane. Standard preparation: An accurately weighed quantity of 50mg Pyrimethamine and 1000mg Sulfadoxine into 200ml volumetric flask, dissolve in and dilute to volume with methanol, sonicate until dissolved. 5ml of resulting solution was transfer to 50ml volumetric flask and dilute to volume with Mobile phase.

Sample preparation: One suppository. (1000 mg Sulfadoxine) was transfer into 200ml volumetric flask Add 100 ml methanol shake well and disperse by heating on a water bath at 70°C for 5 minutes with shaking then sonicate for 5 min dilute to volume cool to room temperature dilute 5ml to 50 ml with Mobile phase, sonicate for 10 minutes. Filter through 0.45µm filter. Procedure: Separately inject equal volumes (20µL) of the standard solution and the sample preparations into the chromatograph, record the area of the major peaks. The percentage of active ingredients was Calculate as following:

$$\text{Assay} = \frac{\text{Area of sample} \times \text{Conc. of std. soln.}}{\text{Area of standard} \times \text{Conc. of sample soln.}} \times \text{purity of std}$$

Assay of Pyrimethamine

Each suppository contains 1000 mg Sulfadoxine and 50 mg Pyrimethamine. HPLC conditions where: Column: C18 5µm 4.6 x 150 mm Flow rate: 2.0 ml / min. Wavelength: 240nm. Sulfadoxine is the first peak and Pyrimethamine is the second peak. Mobile phase: Prepare degassed and filtered mixture of 800ml of 0.01M phosphoric acid and 200ml Acetonitrile, filter by 0.45um Nylon micromembrane. Standard preparation:Weigh an accurately quantity 50mg of Pyrimethamine & 1000 Sulfadoxine into 200ml volumetric flask, dissolve in and dilute to volume with methanol, sonicate until dissolved. Transfer 5ml of resulting solution to 50ml volumetric flask and dilute to volume with Mobile phase. Sample preparation: Transfer 1 suppository (50 mg Pyrimethamine) into 200ml volumetric flask. Add 100 ml methanol shake well. & disperse by heating on a water bath at 70°C for 5 minutes with shaking then sonicate for 5 min dilute to volume cool to room temperature dilute 5ml to 50 ml with Mobile phase, sonicate for 10 minutes. Filter through 0.45µm filter. Procedure: Separately inject equal volumes (20µL) of the standard solution and the sample preparations into the chromatograph, record the area of the major peaks. The percentage of active ingredients was calculated according to the equation mention above.

Then for column washing: only the mobile phase allows to passing through it for 10 minutes.

Assay of Sulfadoxine and Pyrimethamine

Each suppository contains 1000 mg Sulfadoxine and 50 mg Pyrimethamine. HPLC conditions:

Column : C18 5µm 4.6 x 150 mm

Flow rate : 2.0 ml / min.

Wave length : 240nm.

Sulfadoxine is the first peak and Pyrimethamine is the second peak.

Mobile phase: Prepare degassed and filtered mixture of 800ml of 0.01M phosphoric acid and 200ml Acetonitrile, filter by 0.45um Nylon micromembrane.

Standard preparation: Weigh an accurately quantity 50mg of Pyrimethamine and 1000 Sulfadoxine into 200ml volumetric flask, dissolve in and dilute to volume with methanol, sonicate until dissolved. Transfer 5ml of resulting solution to 50ml volumetric flask and dilute to volume with Mobile phase. Sample preparation: Transfer one suppository (1000 mg Sulfadoxine and 50 mg Pyrimethamine) into 200ml volumetric flask. Add 100 ml methanol shake well and disperse by heating on a water bath at 70°C for 5 minutes with shaking then sonicate for 5 min dilute to volume cool to room temperature dilute 5ml to 50 ml with Mobile phase, sonicate for 10 minutes. Filter through 0.45µm filter. Procedure: Separately inject equal volumes (20µL) of the standard solution and the sample preparations into the chromatograph, record the area of the major peaks. The percentage of active ingredients was calculated according to the equation mention above.

Assay of Chloroquine phosphate

Standard preparation

Equivalent weight of Chloroquine phosphate 60mg in to 100ml volumetric flask and complete the volume by 0.1 N HCl and complete the dissolve by sonication. 2ml from above solution was taken in to 100ml volumetric flask and complete the volume with 0.1 N HCl. Sample preparation: 10 supp were melt in water bath until complete melting, then by syringe take equivalent. weight 60 mg into 100 ml volumetric flask and complete the volume by 0.1 M HCl then put in water bath for 30 minutes until complete dissolve then cooling to renormalize volume.

2ml was take into 100ml VF then complete the volume by 0.1M HCl to 100ml. (the final concentration of sample 0.012mg/ml) then reading the absorbance on UV.

Spectrophotometer at 344nm wavelength. The percentage of active ingredients was calculated according to the equation mention above.

Table 10: The Standard Value of All Test According to The British Pharmacopeia.

No	Test	British Pharmacopeia Limit
I	Appearance	
II	Size	2mg
III	yConsistenc	Hard
IV	Color	White for PEG,Witepsol and pale yellow for Cocoa butter
V	Shape	Bullet Shape
VI	Mass Uniformity	±5%
VII	Disintegration	Not more than 30 minutes for fat base. Not more than 60 minutes for water base.
VIII	Dissolution	Not less than 60%
IX	Assay	From 90% to 110%

RESULTS AND DISCUSSION

Melting Point Results

As shown in Table 11 melting point of Pyrimethamine, Sulfadoxine and Chloroquine phosphate.

Table 11: Melting Point Results of Active Ingredients.

Sample	M.P Range (Pharmacopeia)	The Result
Pyrimethamine	239-242°C	240°C
Sulfadoxine	99°C	99°C
Chloroquine phosphate	195- 218°C	207°C

IR Spectroscopy Results

As shown in Figures 1-3 IR of Pyrimethamine, Sulfadoxine and Chloroquine phosphate.

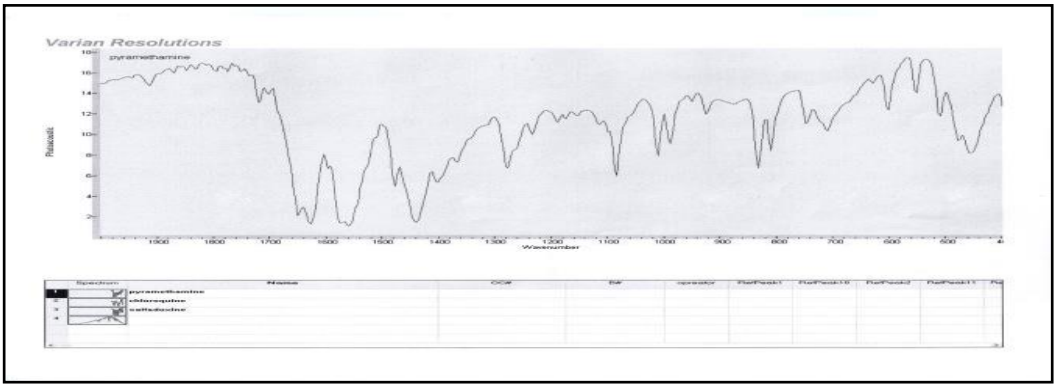

Fig. 1: Pyrimethamine IR Scanning.

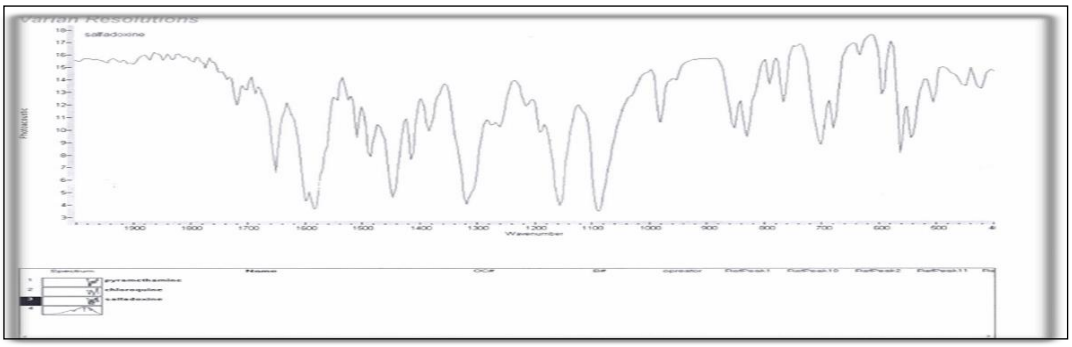

Fig. 2: Sulfadoxine IR Scanning.

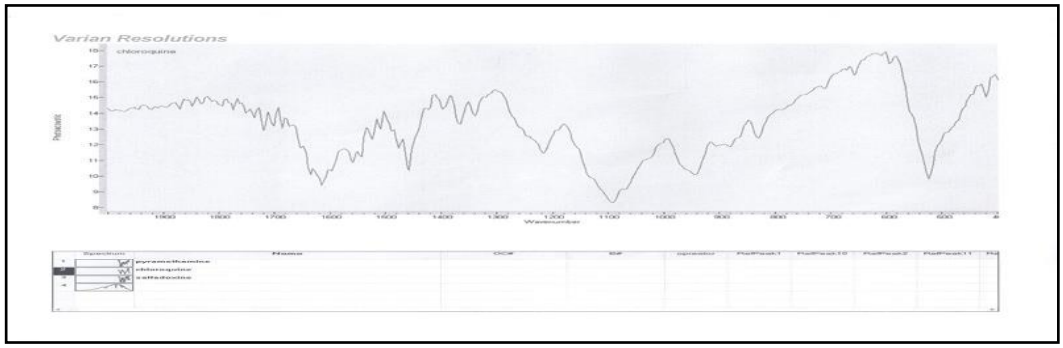

Fig. 3: Chloroquine Phosphate IR Scanning.

Table 12: Physicochemical Parameters of The Suppositories of Pyrimethamine Formulations.

Drug	Type of Base	Shape	Color	Consistency	Disintegration Time	Average Weight
Pyrimethamine (FI)	F1(I)	Bullet Shape	Pale Yellow	Hard Melt	4 min	Within Limit
	F2 (I)	Bullet Shape	White	Hard	6 min	Within Limit
	F3(I)	Bullet Shape	White color	Hard	45 min	Within Limit
	F4(I)	Bullet Shape	White Above & Yellow Bottom	Hard	45 min	Within Limit
	F5 (I)	Bullet Shape	White Above and Yellow Bottom	Hard	5 min	Within Limit
	F6(I)	Bullet Shape	White	Hard	6 min	Within Limit

Table 13: Physicochemical Parameters of The Suppositories of Sulfadoxine Formulations.

Drug	Type of Base	Shape	Color	Consistency	Disintegration Time	Average weight
Sulfadoxine (FII)	F1(II)	Bullet shape	Pale Yellow	Hard Melt	5 min	Within Limit
	F2 (II)	Bullet Shape	White	Hard	6 min	Within Limit
	F3(II)	Bullet Shape	White color	Hard	45 min	Within Limit
	F4(II)	Bullet shape	White Above and Yellow Bottom	Hard	45 min	Within Limit
	F5 (II)	Bullet Shape	White Above and Yellow Bottom	Hard	5 min	Within Limit
	F6(II)	Bullet Shape	White	Hard	6 min	Within Limit

Table 14: Physicochemical Parameters of The Suppositories of Sulfadoxine and Pyrimethamine (Combination) Formulations.

Drug	Type of Base	Shape	Color	Consistency	Disintegration Time	Average Weight
Combination (FIII)	F3 (III)	Bullet Shape	Pale Yellow	Hard melt	45 min	Within Limit
	F4 (III)	Bullet Shape	White	Hard	10 min	Within Limit

Table 15: Physicochemical Parameters of The Suppositories of Chloroquine Phosphate Formulations.

Drug	Type of Base	Shape	Color	Consistency	Disintegration Time	Average Weight
Chloroquine Phosphate (FIV)	F1(IV)	Bullet Shape	Pale Yellow	Hard Melt	4 min	Within Limit
	F2 (IV)	Bullet Shape	White	Hard	6 min	Within Limit
	F3(IV)	Bullet Shape	White Color	Hard	35 min	Within Limit
	F4(IV)	Bullet Shape	White Above and Yellow	Hard	30 min	Within Limit

			bottom			
	F5 (IV)	Bullet Shape	White Above and Yellow Bottom	Hard	5 min	Within Limit
	F6(IV)	Bullet Shape	White	Hard	6 min	Within Limit

Table 16: Drug Release Percent of The Suppositories of Pyrimethamine, Sulfadoxine, Sulfadoxine /Pyrimethamine (Combination) and Chloroquine Phosphate Formulations.

F. Code	Pyrimethamine (FI)	Sulfadoxine (FII)	Combination (FIII)	Chloroquine.P. (FIV)
F1	0.846%	0.798%	-----	1.962%
F2	0.96%	2.964%	-----	58.716%
F3	2.088%	35.22%	%9.264 =.Pyri Sulf.=14.304%	76.8%
F4	10.8%	4.722%	Pyri= 0.56% Sulf.= 0.822%	14.31%
F5	1.512%	0.564%	-----	49.032%
F6	1.158%	0.102%	-----	2.586%

Table 17: Drug Assay of The Suppositories of Pyrimethamine, Sulfadoxine, Sulfadoxine /Pyrimethamine (Combination) and Chloroquine Phosphate Formulations.

F. Code	Pyrimethamine (I)	Sulfadoxine (II)	Combination (III)	Chloroquine.P. (IV)
F1	99.57%	75.48%	-----	100.1%
F2	85.7%	70.77%	-----	99.63%
F3	111.12%	79.8%	Pyri=117.28% Sulf= 67.56%	99.78%
F4	109%	62.13%	Pyri= 73.93% Sulf=56.21%	99.58%
F5	75%	46.09%	-----	97.84%
F6	133%	6.62%	-----	95.387%

As shown in Tables 12 to 15 all Pyrimethamine, Sulfadoxine, sulfadoxine and pyrimethamine (combination), and Chloroquine phosphate suppositories show an acceptable uniformity of weight according to British pharmacopeia specification. The significance of this test is to ensure that the suppositories are within the appropriate size range.

As shown in Tables 12 to 14 for Pyrimethamine, Sulfadoxine and sulfadoxine /pyrimethamine (combination) suppositories passed the disintegration test and formulations F3(I), F4(I) and F3(III) takes 45 min. while for Chloroquine phosphate suppositories F3(IV) and F4(IV) takes 35 and 30 min respectively as shown in Table 15.

Table 10 show the standard value for all tests according to the British Pharmacopeia and Table 11 show that all material used pass the melting point as compare to the Pharmacopeia.

The dissolution results as shown in Table 16 all formulations of Pyrimethamine, Sulfadoxine, sulfadoxine and pyrimethamine (combination), and Chloroquine phosphate suppositories did not pass the test according to the pharmacopoeia except formula F3(IV) (Chloroquine phosphate with PEG4000) which passed the Pharmacopeia limit.

As illustrated in Table 17, the assay of all Chloroquine phosphate suppository formulations was within the acceptable limit, for Pyrimethamine all formulations (F1, F3, F4 and F6) were pass the Pharmacopeia limit except (F2 and F5) were not pass, while Sulfadoxine, sulfadoxine and pyrimethamine (combination) formulation suppositories were not pass.

Form the above results PEG base show the best release for Pyrimethamine, Sulfadoxine, sulfadoxine and Pyrimethamine (combination), and Chloroquine phosphate, while cocoa butter and Witepsol H35 show the lowest drug release.

The formulations failed in drug release test may be due to the fact that the disintegration particles are not small enough to pass through the screen of the dissolution basket and retained the active drug within their hard coarse and hence did not release the drug into the dissolution medium, this implies that the product may not release a significant amount of the drug on absorption in to the systemic circulation, this leading to therapeutic failure.

The formulations which do not pass the assay test, may be due to poor preparation techniques during formulation and subsequent manufacturing, furthermore the amount of Pyrimethamine in combination suppositories is relatively small which means any dismissing or segregating during processing will result in a non-uniformity of drug content.

Also, it may be that, the natural of Pyrimethamine or Sulfadoxine cannot dissolve in the media selected and therefore did not get liberated during the analysis process.

CONCLUSION

The prepared batches of antimalarial drugs suppositories were evaluated for physicochemical parameters, weight variation, disintegration time, and in-vitro drug release. It was concluded that the disintegration test results of all formulated suppositories are within the acceptable limit. The best results show in PEG4000 base according to percent of drug release of

Chloroquine phosphate, Pyrimethamine, Sulfadoxine, Sulfadoxine and Pyrimethamine (combination) formulations, while cocoa butter and Witepsol H35 show the lowest drug release. Assay results of all formulations of Chloroquine phosphate and three formulations of Pyrimethamine are within the acceptable limit. Among the all formulations F3(IV) Chloroquine phosphate formulation was found to be showed improved drug release characteristics. F3(IV), which consists of (Chloroquine phosphate and PEG4000 base), showed the best result in drug release and higher efficiency values while Cocoa butter and Witepsol H35 base are less release for antimalarial drugs in formulated suppositories.

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