The Stability of Folic Acid Suspension

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***Abstract*- INTRODUCTION AND OBJECTIVES**

This study was to investigate the physicochemical and microbiological stability of an extemporaneous oral suspension1mg/ml of folic acid.

METHODOLOGY

Folic acid suspension was prepared according to hospital formulation. The oral suspensions were divided into bottles labeled A,B,C stored at 25°C (room temperature) and bottled labeled E,F,D and stored at 4°C (refrigerated). The suspension was then scored for changes in visual appearance, odor, pH and microbial growth on Day 0, 7,14,30,60 and 90.

RESULTS

The folic acid suspension retained its colour and opaquecity from day 0 to day 90. There were no visible changes in colour or odour of folic acid suspension throughout the study. The samples pH was constant from day 0 to day 60 at pH 5.0. However the pH was deranged at day 90. pH of sample A and sample C which was stored at 25°c increased from pH 5.0 to pH 5.5 and 6.0 respectively while only 1 sample E stored at 4°c increased from pH 5.0 to pH 5.5 . The test scored positive once out of 108 samples tested for microbial growth which is sample F (store 4°c) on Sabauroud dextrose agar for test on day 7.

CONCLUSION

This study have shown that this formulation of extemporaneously prepared folic acid oral suspensions stored at 4°C (refrigerated) and 25°C (room temperature) is stable up to 60 day supported by the pH and physiochemical result . It is recommended that the shelf life of this formulation is to be 60 days and storage condition of 4°C (refrigerated).

***Index Terms*-** folic acid , formulation , extemporaneous , stability

I. INTRODUCTION

An extemporaneous preparation is defined as a "drug or combination of drugs prepared or compounded in a pharmacy according to a prescription" (Section 1(1) of O.Reg 201/96 made under the Ontario Drug Benefit Act). Extemporaneous preparation is important to suit individual patient's need especially pediatric patient population [1.]

Solid dosage forms medication present problems as pediatric patients have difficulty swallowing whole tablets or capsules. In absence of availability of liquid formulation, extemporaneous preparations are often used. Problem linked with extemporaneous formulations highlighted as unknown expiry date of extemporaneous preparation.

Folic acid oral suspension is one of the commonly prepared extemporaneous oral preparations in hospital pharmacies[1.] Folic acid has been used as folate supplement in children who have anemia (BNF for children, 2009). However, the information related to the extemporaneous formulations and the stability of the folic acid is lacking[2]

No specific formula for folic acid oral suspension available in MOH Formulary and no commercial product for folic acid oral suspension available in Malaysia[3,4] . Furthermore, no standard expiry dates available in dispensing folic acid oral suspension. The dispensing of folic acid differs between hospital setting with some hospital practice expiry date between 2 week or 1 month. There are some hospitals that supply tablets with patient instructed to dilute the medication prior to administration.

This study is designed to study the stability of folic acid 1mg/ml solution as 1mg/ml is the solution concentration prepared at MOH hospitals. It is common practice by prescriber to calculate the dose based on 1mg/ml strength. Furthermore it is convenient to administer 1mg/ml volume to the neonate/pediatric patient.

Stability of a solution is determined by changes in physiochemical properties namely changes in pH, visual (color/sedimentation) and odor of all which is tested in this study.

1.2 Research Objectives

1.2.1 General

To investigate the physicochemical and microbiological stability of an extemporaneous oral suspension1mg/ml of folic acid.

1.2.2 Specific

i. To study the stability difference between extemporaneously prepared folic acid oral suspensions stored at 4°C (refrigerated) and 25°C (room temperature).
ii. To determine the shelf life and storage condition of the extemporaneous oral suspension.
iii. To evaluate the pH and appearance of folic acid oral suspension throughout the study period.

II. LITERATURE REVIEW

2.1 Use of Folic acid in pediatric patient

Folic acid is a component of the B group of vitamins and is necessary for the normal production and maturation of red blood cells. It has been use for the treatment of folate-deficient megaloblastic anemia due to malnutrition, malabsorption syndromes (such as coeliac disease) and increased utilization as

in pregnancy. In woman planning for pregnancy, folic acid was also used as prevention of neural tube defect [5].

For young children a more suitable dosage form should be used. The dose for folate deficient megaloblastic anemia is 5mg daily for 4 months and the maintenance is 5mg every 1-7 days. For hemolytic anemia and metabolic disorders the dose is 2.5mg-5mg once daily [6].

It is common practice to prescribe pediatric patient with folic acid suspension as vitamin supplement for general wellbeing.

2.2 Practices and Problems associated with Preparation of Oral Liquids

2.2.1Chemical instability

Drugs in extemporaneously prepared liquids may be susceptible to chemical reactions leading to degradation. The most common reactions are hydrolysis, oxidation and reduction. Usually the reaction rate and type is influenced by solution pH. Preparations made from tablets contain excipients such as binders and disintegrating agents in addition to the active drug. These excipients may reduce chemical stability by changing the pH to a value at which more rapid degradation occurs[7]

A study by Manzur UI [8] has shown that folic acid is stable in aqueous solution between pH 5 and 8. On the other hand, Dick and Coworker[9] reported that negligible decomposition occurs on autoclaving folic acid solution for 15 minutes at 121°C at pH 5 to 10.

2.2.2 Microbiological Instability

Microbial growth in an oral liquid may cause foul odor and turbidity and adversely affect palatability and appearance. High titrates of micro-organisms may be hazardous to health especially in very young or immunocompromised patients. By-products of microbial metabolism may cause a change in the pH of the preparation and reduce the chemical stability or solubility of the drug. Consequently the drug must also be stable at this pH. Effective preservative systems require rigorous evaluation which is seldom performed on extemporaneous formulations [7]

2.2.3Physical Instability

Extemporaneously prepared oral suspensions may be susceptible to sedimentation of insoluble drug causing caking. Difficulty in re-suspending the drug or rapid sedimentation following shaking can lead to erratic dosage measurement and this inherent problem with extemporaneously prepared formulations is of considerable concern. Refrigeration, whilst usually desirable to maximize chemical stability and reduce microbial growth, can also increase the viscosity of a suspension making re-suspension more difficult or cause the precipitation of active drug or preservatives. It is important to consider the effect on pH of all components of the formulation and the possible impact on stability [7]

III. MATERIAL AND METHODOLOGY

3.1 Commercial Drug and Vehicle

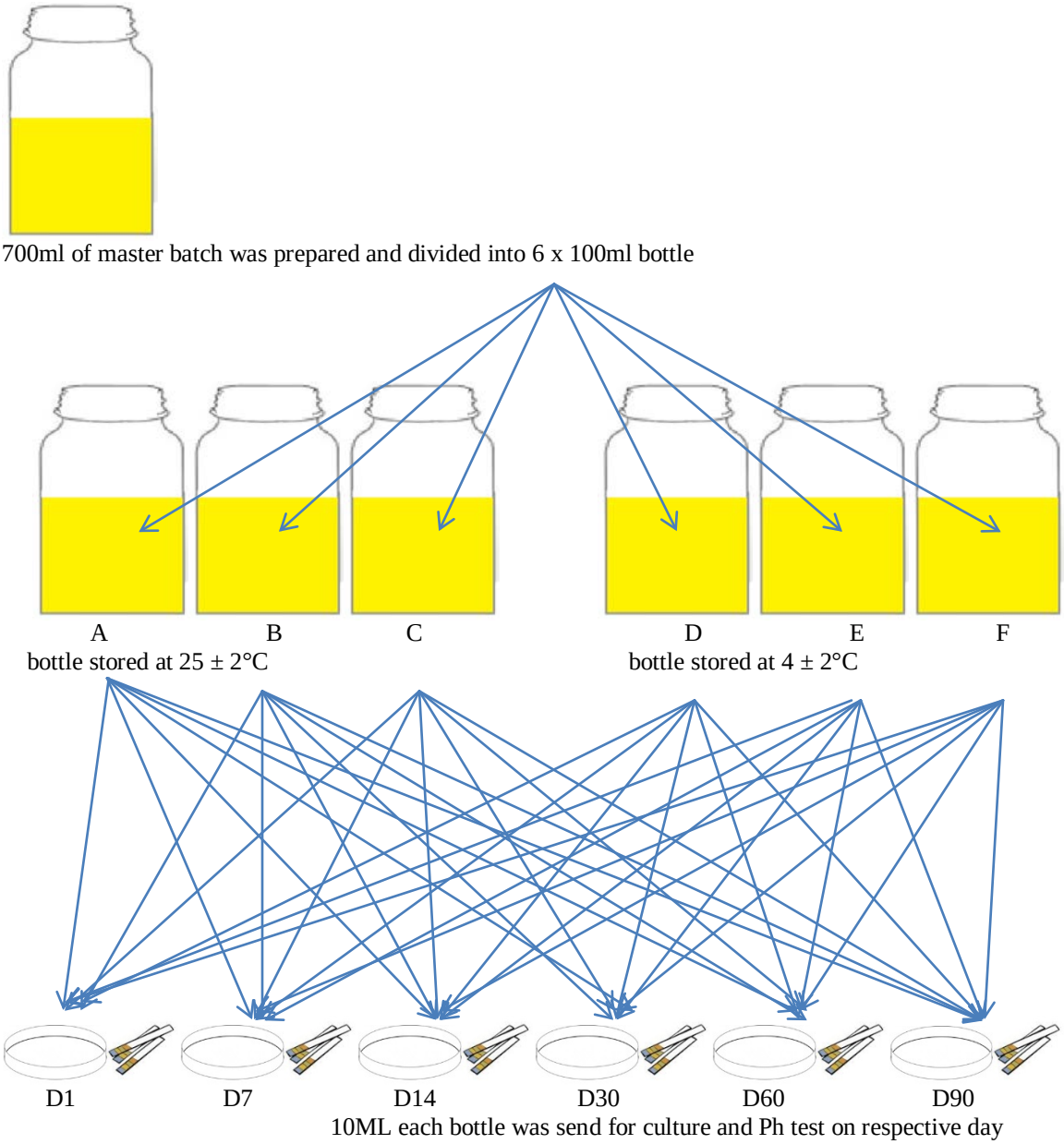
Tablets containing 5mg of folic acid manufactured by Duopharma (Batch number: 131160, Expired: November 2016) were used for the compounding of folic acid oral suspension. The vehicle used is syrup Simplex manufactured by KCK Pharmaceutical Industries Sdn Bhd (Batch number: L1405011 Expired: May 2016).

3.2 Extemporaneous preparation

Ground 140 tablet of 5mg folic acid using a mortar and pestle to form fine powder. Gradually add syrup simplex to levitate the fine powder. Continue mixing of powder and syrup simplex in the mortar until uniform paste is formed. Transfer the paste into a graduated conical flask. Rinse the mortar and pestle with syrup simplex and transfer the solution into graduated conical flask. Repeat the rinsing process until all the folic acid paste is transferred to the graduated conical flask. Add 14 milliliter of Raspberry essence into the graduated conical flask. Syrup Simplex was added into the graduated conical flask up to total of 700 milliliter. The suspension was stirred with a glass rod until a uniform solution was formed.

Pour 100 ml of 1mg/ml folic acid oral suspension into 6 bottles of 120ml semi- transparent plastic bottles. Wrap the bottle with aluminum foil. Label 3 bottles as A, B and C and store at 25 ± 2°C (room condition). Label the other 3 bottles as D, E and F and store at 4 ± 2°C.

Figure 1 : Packing of Folic Acid Suspension



3.3 Physicochemical Stability Studies

Examine 1mg/ml folic acid suspension on Day 0, 7, 14, 30, 60 and 90 for changes in visual appearance, odor, Ph. and microbial contamination.

3.3.1 Visual appearance Study

Examine the sample for changes in visual appearance. Score positive (+) for changes in visual Appearance and negative (-) for no changes in visual appearance.

Table 1: Visual appearance of folic acid oral suspension

Time (Days)		0	7	14	30	60	90
25°C	A						

	B						
	C						
4°C	D						
	E						
	F						

3.3.2 Odor Study

Examine the sample for changes in odor. Score positive (+) for unpleasant odor and negative (-) for pleasant odor.

Table 2: Odor of folic acid oral suspension

Time (Days)		0	7	14	30	60	90
25°C	A						
	B						
	C						
4°C	D						
	E						
	F						

3.3.3 pH Study

Transfer 5ml of 1mg/ml folic acid suspension from each bottle into a respectively labeled container .The samples pH will be measured by the Pathology department unit, Hospital Seri Manjung.

Table 3: pH of folic acid oral suspension

Time (Days)		0	7	14	30	60	90
25°C	A						
	B						
	C						
4°C	D						
	E						
	F						

3.3.4 Microbiological Stability Studies

The microbial test will be carried out by the Pathology department unit, Hospital Seri Manjung. Transfer 5ml of 1mg/ml folic acid suspension from each bottle into a respectively labeled container. Pathology lab will subculture the sample into blood agar, MacConkey agar and Sabauroud dextrose agar and incubates for 24-72hours. Score positive (+) for growth and negative (-) for growth absence.

Table 4: Microbial results of folic acid oral suspension

Time (Day)		0	7	14	30	60	90
Blood agar	A						
	B						
	C						
	D						
	E						
	F						
MacConkey agar	A						
	B						
	C						
	D						

	E						
	F						
Sabauroud dextrose agar	A						
	B						
	C						
	D						
	E						
	F						

3.4 Ethical consideration

Ethical approval will be obtained from the National Medical Research and Ethics Committee (MREC) of Ministry of Health (MOH) via the National Medical Research Register (NMRR).

3.5 Data analysis

The physicochemical, microbiological and analytical properties of extemporaneously prepared folic acid oral suspension will be presented as descriptive analysis.

IV. RESULT AND DISCUSSION

4.1 Result of Physicochemical Stability Studies

4.1.1 Result of Visual appearance study

The suspension was scored positive (+) for changes in visual appearance and negative (-) for no changes in visual appearance. Photographic evidence was taken on day 0, 7, 14, 30, 60 and 90. The suspension retained its color and opaquecity from day 0 to day 90(picture 1 - picture 6) .There was no visible changes in color of folic acid suspension throughout the study. There was visible accumulation of fructose crystal in the neck of the bottle and the cap (picture 7-picture 8) .The formation of the crystal is due to the dehydration of the residual of the suspension at this site**.**

Table 5 : Visual appearance of folic acid oral suspension

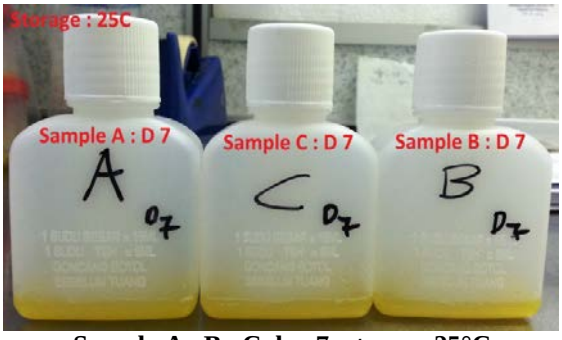
Time (Days)		0	7	14	30	60	90
25°C	A	-	-	-	-	-	-
	B	-	-	-	-	-	-
	C	-	-	-	-	-	-
4°C	D	-	-	-	-	-	-
	E	-	-	-	-	-	-
	F	-	-	-	-	-	-

Picture 1

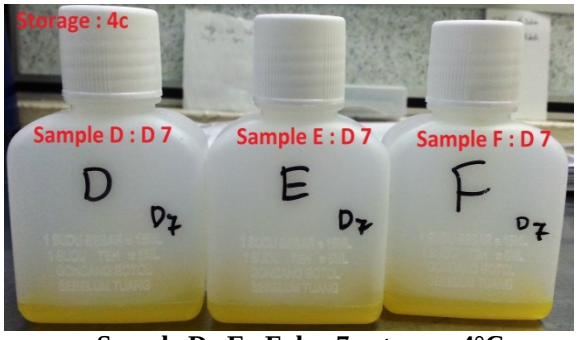
Sample A , B , C day 1: storage 25°C

Sample D , E , F day 1 : storage 4°C

Picture 2

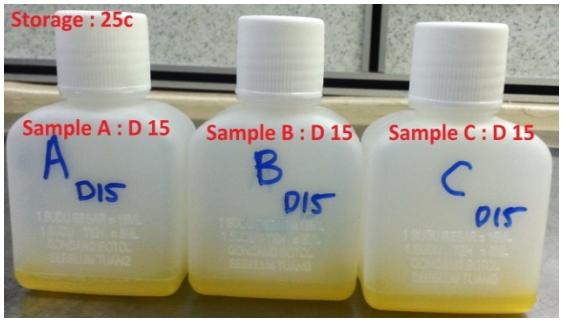


Sample A , B , C day 7: storage 25°C

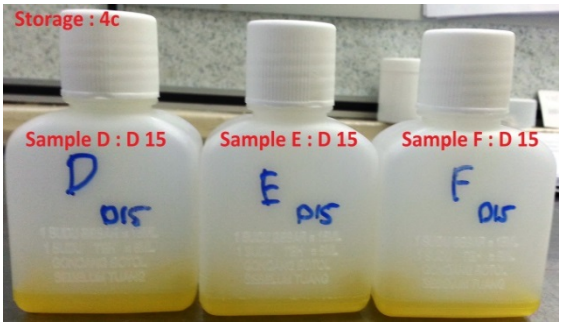


Sample D , E , F day 7 : storage 4°C

Picture 3



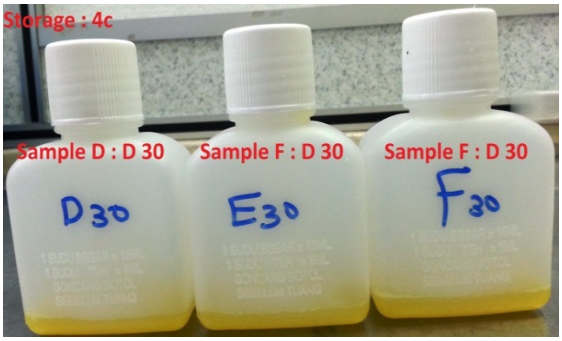
Sample A , B,C day 14: storage 25°C



Sample D , E , F day 14 : storage 4°C

Picture 4

Sample A , B,C day 30: storage 25°C



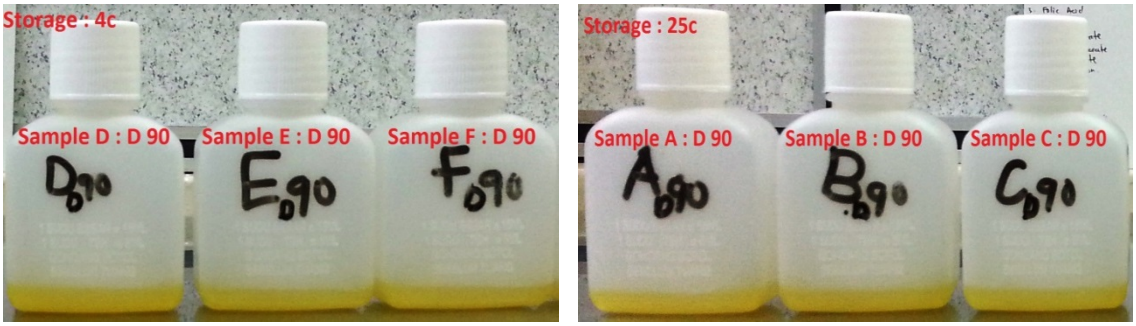
Sample D , E ,F day 30 : storage 4°C

Picture 5

Sample A , B,C day 60: storage 25°C

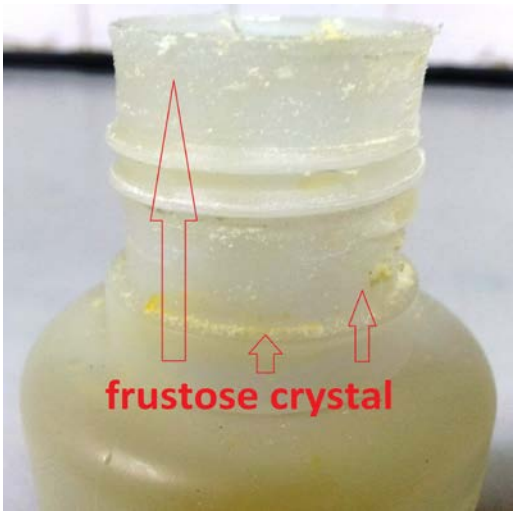
Sample D , E , F day 60 : storage 4°C

Picture 6



Sample A , B,C day 90 : storage 25°C **Sample D, E ,F day 90 : storage 4°C**

Picture 7



Picture 8

Formation of fructose crystal in the neck and on the cap of the bottle

The Suspension was examined for changes in odor. The suspension was scored positive (+) for unpleasant odor and negative (-) for pleasant odor.

4.4.2 Odor Study

Table 6: Odor of folic acid oral suspension

Time (Days)		0	7	14	30	60	90
25°C	A	-	-	-	-	-	-
	B	-	-	-	-	-	-
	C	-	-	-	-	-	-
4°C	D	-	-	-	-	-	-
	E	-	-	-	-	-	-
	F	-	-	-	-	-	-

The suspension was smelled sweet raspberry throughout the study period. No changes in odor were noticed up to day 90 of the study. No changes in odor indicate the formulation will be palatable for 90 days and there was no bacterial growth in the formulation.

4.4.3 pH Study result

Table 7: pH of folic acid oral suspension

Time (Days)		0	7	14	30	60	90
25°C	A	5.0	5.0	5.0	5.0	5.0	5.5
	B	5.0	5.0	5.0	5.0	5.0	5.0
	C	5.0	5.0	5.0	5.0	5.0	6.0
4°C	D	5.0	5.0	5.0	5.0	5.0	5.0
	E	5.0	5.0	5.0	5.0	5.0	5.5
	F	5.0	5.0	5.0	5.0	5.0	5.0

The samples pH was measured by the Pathology department unit, Hospital Seri Manjung. The samples pH was constant from day 0 to day 60 at pH 5.0. However the pH was deranged at day 90. pH of sample A and sample C which was stored at 25°c increased from pH 5.0 to pH 5.5 and 6.0 respectively while only 1 sample E stored at 4°c increased from pH 5.0 to pH 5.5 .

The derangement in pH might be due to the formation of fructose crystal in the neck and cap of the bottle (picture 7, picture 8). The Crystal might have changed the equilibrium of the suspension and hence the changes in pH.

A study by Manzur UI[8] has shown that folic acid is stable in aqueous solution between 5 and 8. This is further supported by a study by Ester P Daniel[10], reported that negligible decomposition occur on autoclaving folic acid solution for 15 minutes at 121°C at pH 5 to 10. A recent study done in 2012 showed that liquid preparation was stable at pH range of 5 to 5.5 and no significant degradation when the liquid was stored for 2 years at room temperature[11]

Although there was derangement in pH, it is safe to say that the solution was stable and safe for consumption.

4.4.4 Microbiological Stability Studies

The microbial test was carried out by the Pathology department unit, Hospital Seri Manjung. The sample was subculture by the Pathology lab into blood agar, MacConkey agar for 24 hour and Sabauroud dextrose agar for 72hours. The result was score positive (+) for growth and negative (-) for growth absence. The test for total microbial count was not carried out due to lack of suitable equipment and no budget given.

Table 8: Microbial results of folic acid oral suspension

Time (Day)		0	7	14	30	60	90
Blood agar	A	-	-	-	-	-	-
	B	-	-	-	-	-	-
	C	-	-	-	-	-	-
	D	-	-	-	-	-	-
	E	-	-	-	-	-	-
	F	-	-	-	-	-	-
MacConkey agar	A	-	-	-	-	-	-
	B	-	-	-	-	-	-
	C	-	-	-	-	-	-
	D	-	-	-	-	-	-
	E	-	-	-	-	-	-
	F	-	-	-	-	-	-
Sabauroud dextrose agar	A	-	-	-	-	-	-
	B	-	-	-	-	-	-
	C	-	-	-	-	-	-
	D	-	-	-	-	-	-
	E	-	-	-	-	-	-
	F	-	+	-	-	-	-

The test was only scored positive once for sample F (store 4°c) for test on day 7. The growth on Sabauroud dextrose agar indicated the growth of dermatophytes and fungi. Subsequent test on the same sample failed to score for any growth. It is sufficient to say that the inoculate might be due to exposure to air or contamination during the transferring or sampling of the folic acid suspension.

V. Study Limitation

The limitation of this study is lack of analytical data to support our result. The content folic acid in the folic acid suspension needs to be measured by HPLC-UV method to

quatitify the amount of folic acid through the study period. Although we could establish the stability of the suspension, we could not establish the content of folic acid in the formulation

However a recent 2012 study by Vignesh M, have shown that folic acid suspension was able to maintain the concentration of folic acid in the suspension above 90 % up to 6 month at room temperature when the pH is 5.3[11.] Another study by Lucy Y, have shown that the concentration of folic acid assay is more than 90% for 60 days at pH between 3.3 to 5.3 [12] .

The microbial stability test method was not done according to the British Pharmacopeia 2010 for non-sterile product.

This limitation is due lack of access to the laboratory equipment to require to run the study according to internationally accepted standard .The limitation above will be concern if this formulation to be used to produce folic acid suspension for commercial purpose. Since this formulation is for extemperonueos preparation of folic acid suspension, the data could be used as a reference to determine the expiry date of the suspension prepared extemporaneously in hospital setting .

VI. Future consideration

This study should adopt method recommended by British pharmacopeia 2010 for non-sterile product

VII. Recommendation

A commercially available suspending system "X-Temp oral suspension" could be used to prepare folic acid suspension. A study with Folic acid suspension prepared with X-temp oral suspension has been shown to be stable for 60 days. The method used in the study conforms to British pharmacopeia 2009 standard.

However, cost to use X-temp will be a concern. A study on cost analysis of using syrup simples versus X-temp as suspension vehicle has shown that it cost RM 0.23 to produce 30ml of folic acid suspension using syrup simplex and RM 3.08 to produce 30ml of folic acid suspension using X-temp[13]

VIII. Conclusion

This study have shown that this formulation of extemporaneously prepared folic acid oral suspensions stored at 4°C (refrigerated) and 25°C (room temperature) is stable up to 60 day supported by the pH and physiochemical result . It is recommended that the shelf life of this formulation is to be 60 days and storage condition of 4°C (refrigerated).

Acknowledgement

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