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Folic Acid Granulation and Evaluation for Pediatric Diseases

Baha Majdi Abd Alrahman Sabri

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FOLIC ACID GRANULATION AND EVALUATION FOR PEDIATRIC DISEASES

Prepared by:

Baha Majdi Abd Alrahman Sabri

B.Sc. Chemistry, Al-Quds University, Palestine

Supervisor: Prof. Ibrahim Kayalli

Co-Supervisor: Dr. Mahmoud Al-khatib

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Applied Industrial Technology Program

Thesis Approval

Prepared by Baha Majdi Abd Alrahman Sabri
Registration No.: 21610402
Supervisor: Prof. Ibrahim Kayali
Co-Supervisor: Dr. Mahmoud Al khatib

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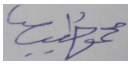
The names and signatures of the examining committee members are as follows:

1-Head of Committee: Prof. Ibrahim Kayali Signature: 

2- Co-Supervisor: Dr. Mahmoud Al khatib Signature: 

3- Internal Examiner: Dr. Wadie Sultan Signature: 

4- External Examiner: Dr. Fahed Takrori Signature: 

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Dedication:

I want to dedicate this work to my father soul, for my loved mother, brothers, and sister
My great family and all supportive friends for their love, endless support, encouragement.
Moreover, sacrifices& Al-Quds University staff and students, who have given us wisdom & knowledge, insistence and patience.

Declaration:

I certify that the thesis submitted for the degree of a master is the result of my research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not to be submitted for a higher degree to any other university or institution.

Signed: 

Name: Baha Majdi Abd Alrahman Sabri

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Abstract

Folic acid is water-soluble vitamin. It is an important compound involved in many necessary biochemical processes in humans. It is broadly used to treat many diseases of pediatric like megaloblastic anemia and neural tube defects. The lack of suitable folic acid dosage form for children creates a need to cover this category.

This research aims to develop a new formula of folic acid that suits pediatric needs and study the effect of heat, light, and pH on specific formula.

Three different formulas of folic acid were prepared as granules by using two methods dry and wet granulation. HPLC was used to determine folic acid concentration during the stability study period. UV-visible spectrophotometer was used to measure the absorbance of folic acid after exposure to UV light at different pH in order to study the extent of degradation.

Results showed wet formula reached to 97.84% of the concentration of folic acid at the beginning, but after six months at stress condition (40°C ± 2°C& 75% RH ± 5%), it reaches 70.46%. On the other hand, the results of dry formula at the beginning reached 105.30% of the concentration of folic acid. Also, after six months at stress condition (40°C ± 2°C& 75% RH ± 5%) reached 97.00%.

The results of the effect of light and pH showed folic acid at acidic medium (pH= 1.47 and 5.50) to have degradation actions, the folic acid lost (16% and 8%), respectively, of its concentration after two hours of exposure to 365 nm light. However, folic acid at basic medium (pH= 8.36 and 10.60) had different actions; the loss of concentration of folic acid reached (3.5% and 2.6%), respectively, after two hours of exposure to 365 nm light.

The presence of water in the wet granulation method increased the rate of degradation.
The acidic medium increased the cleavage of the structure of folic acid in addition to the illumination.

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List of abbreviations:

USP: United States Pharmacopeia.

WHO: World Health Organization.

ICH: The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use.

MOH: Ministry of Health.

RH: Relative Humidity.

Hb: Hemoglobin.

API: Active pharmaceutical ingredients.

Chapter One

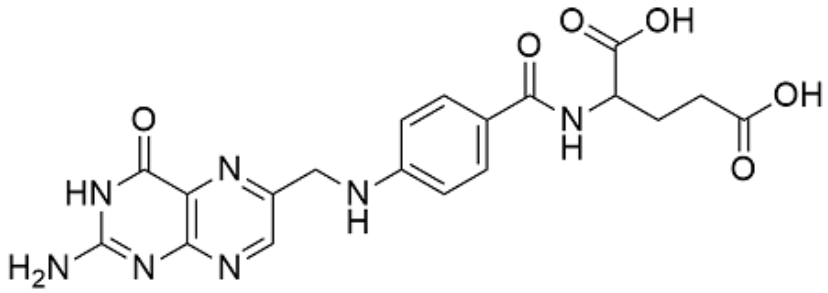
INTRODUCTION

1.1 Folic acid

Folic acid is a water-soluble vitamin. It is an important compound involved in many necessary biochemical processes in humans, mostly in its ionic form. It is included in the production of red and white cells and supports the synthesis of chemicals that adjust brain function. The folate is available in natural sources like leafy green vegetables, pulses, liver, yeast, citrus fruits, nuts, and available in the synthetical form. (CWittho 2016). Folic acid (pteroyl mono glutamic acid, or PGA) is the most oxidized and stable form of folate. It is the form used in vitamin supplements and fortified food products. (Amirah, et al. 2016). Folic acid, known as folate or folacin, is a member of the B-complex family of vitamins and works in concert with vitamin B12.It is functioning primarily as a methyl-group donor involved in many essential body processes, including DNA synthesis. (JW Miller 2013).

1.1.1Biochemistry of folic acid.

Folic acid is composed of three primary structures, a hetero-bicyclic pteridine ring, para-aminobenzoic acid (PABA), and glutamic acid.
It is a dietary requirement because humans cannot synthesize this compound.



Folic acid

Figure 1. 1: Folic acid structure (DK Hansen et al. 2014).

1.1.2 Bioavailability of folic acid.

Folic acid used as a supplement separate from food is most highly bioavailable. The bioavailability of folic acid taken with a meal or as a food fortification is roughly 85% compared with folic acid consumed while fasting.

Natural food folates are even less bioavailable at approximately 50%of the value for folic acid alone. (JW Miller 2013).

1.1.3 Therapeutic indications of folic acid.

Folic acid is necessary for the average production and maturation of red blood cells. It is indications:

1. For the treatment of folate-deficient megaloblastic anemia due to malnutrition, malabsorption syndromes, and increased utilization as in pregnancy. It should not be utilized alone in undiagnosed megaloblastic anemia, including in infancy, pernicious anemia, or macrocytic anemia of unknown an etiology.
2. For the prevention of drug-induced folate deficiency, e.g., caused by administration of phenytoin, phenobarbital, and primidone.
3. For the prophylaxis against folate deficiency in chronic hemolytic states or renal dialysis.
4. For the prevention of neural tube defects for women planning a pregnancy and known to be at risk.

1.1.4 Pharmacokinetic properties of folic acid.

Absorption – folic acid is rapidly absorbed from the gastrointestinal tract, mostly from the proximal part of the small intestine. The naturally occurring folate polyglutamate is largely deconjugated and reduced by di hydro folate reductase in the intestine to form 5-methyltetrahydrofolate (5MTHF). Folic acid provided therapeutically enters the portal circulation, mostly unchanged since it is a poor substrate for reduction by dihydrofolate reductases. (JW Miller 2013).

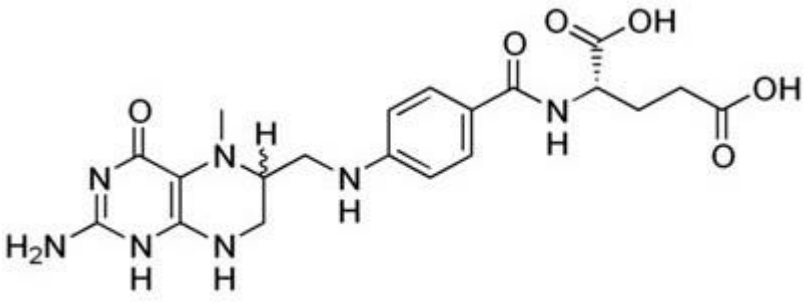


Figure 1. 2: The structure for 5MTHF (Francesco Scaglioneet al. ,2014)

Distribution via the portal circulation. 5MTHF from naturally occurring folate is extensively plasma bound. The leading storage site of folate is in the liver. Folate is distributed into breast milk. (Martin A. Hofsäss et al. ,2017).

Metabolism – therapeutically provided folic acid is converted into the metabolically active form 5MTHF in the plasma and liver. There is an enterohepatic circulation for folate. (James A. Greenberg et al., 2011).

Elimination – Folate metabolites are removed in the urine, and folate more than body requirements is excreted unchanged in the urine. Folic acid is removed by hemodialysis. (JW Miller 2013).

1.2 Dosage form

The great challenge faced researchers and companies in providing a new drug to cover the deficiency of drugs to fight diseases, especially for children. Children had several aspects that made them different than adults in pharmacokinetics and pharmacodynamics, including capabilities for drug administration, medicine associated toxicity, and taste preferences. When formulated, pediatric medicines take into consideration that drugs should fit children's needs, age, physiological condition, body size, and treatment requirements. More concern to provide sufficient treatment of all children, various routes of administration, dosage forms, and strengths may be needed. (Verica Ivanovska 2014, WHO,2012).

The pharmaceutical industry should improve pediatric formulations, and the information on the critical to quality aspects of pediatric medicines is supposed to increase quickly;(European Medicines Agency, 2011).

Pediatric drug development relates to numerous challenges, including methodological and ethical conditions for pediatric trials, high developmental prices, and a small and fragmented store. The pediatric market has focused frequently on only an insufficient number of therapeutic areas, like anti-infective, hormones, and medicines for the respiratory and central nervous system. (Verica Ivanovska et al. 2014).

This lack of pediatric formulations often leaves health care professionals no alternative but to use adult medicines in an off-label or unlicensed manner, the use of unlicensed and off-label drugs for treating children is widespread. In the European Union, 45% to 60% of all medicines are provided to children off-label. This trend is also actual for 90% of drugs administered to neonates and infants. Besides, in the United States, two-thirds of medicines used in pediatrics are off-label; worldwide, this proportion is up to three quarters. (VericaIvanovska et al. 2014).

The effects of such an adults formulations on children have not been appropriately investigated, age-appropriate formulations are generally not available, and the medicines are not licensed for use in children. (WHO,2012).

Pharmacists, parents, or caregivers have usually faced problems to provide a dose for children, that creates needs to manipulate for the adult drug without a clear procedure on how to prepare it for children.

(WHO,2012). This manipulation can be simple, e.g., breaking tablets that do not have a scoreline with a tablet splitter or complex, e.g., utilizing tablets as a source for an active pharmaceutical ingredient (API) to prepare a suspension. (WHO,2012).

The manipulation process itself can increase the potential time consuming, can be inaccurate dosing and in common can increase the variability of the product, and have

effects on the stability, bioavailability, and accuracy of the dosing of the drug. The use of such medicines could expose children to overdosing and unintended side-effects or underdosing and a resultant reduction in value. Likewise, excipients that are safe for adults may not necessarily be so for children. (WHO,2012, Roberta H Richey et al., 2013).

The standard dosage form used with children is oral administration; it can be accomplished through several types of dosage forms. In general, the main option is between the application of oral liquid preparation, a solid oral unit dosage form (e.g., normal-sized tablet, capsule), or an oral flexible solid dosage form (e.g., powder, granules, pellets). (European Medicines Agency, 2011).

Oral liquid preparations involve aqueous solutions, suspensions, emulsions, and syrups. They are most suitable for children in the youngest age groups who are powerless to swallow solid dosage forms. The advantage of oral liquid preparations is that maximum dosage flexibility, ease of eating, the opportunity to flavor as required, and single or multiple-use packs. (Tom Sam et al. 2012) (WHO,2012).

The disadvantage of the liquid oral necessity for stabilizing agents, e.g., antimicrobial preservatives, limited of chemical instability, insufficient control overdose intake, limited shelf life, and limited solubility requiring pH buffers use of co-solvents which may guide to a requirement for controlled storage conditions through distribution and use. Oral liquid preparations are less transportable than solid-dose developments because of their moderately high bulk volume. (Tom Sam et al. 2012) (WHO,2012).

Oral solid dosage forms are useful, economical, and user-friendly, featured with excellent stability, accurate dosage, small packing size, and easy administration. However, sometimes, difficulties of swallowing may exist.

So, a flexible formulation can be designed to combine the advantages of each of the two methods mentioned above. A flexible formulation can be pellets, powders, or granules that can be filled into sachets or capsules and can be dispersed in water or soft foods such as yogurts or apple juice before administration. (WHO,2012).

The lack of the availability of the oral administration of medicines pediatrics includes folic acid. There is no desired dosage form for the pediatric target category. This is related to the shortage in the stability of folic acid in the liquid solution containing water, glycerin, sorbitol, and propylene (Vignesh et al. 2012. On the other hand, solid dosage form increases the stability of folic acid (Awab et al. 2016) (Larry et al. 2008), but it is not palatable to provide the tablet for children.

To determine the optimal formulation of folic acid, several combinations of fillers disintegrates, and binders were estimated, as well as two preparing methods wet granulation by the high-shear mixer and dry mixing for direct compression were used. (Ljiljana Krsteska et al. 2011).

Granulation is one of the highly important processes in the manufacturing of pharmaceutical solid oral dosage forms. Granulation is performed to enhance the characteristics of starting materials, like flow properties, tablet ability, and bulk density. (Oscar-Rupert Arndt et al. 2018). Granulation is defined as the process of agglomeration of a dry powder mixed with a suitable binder. (Dilip M. Parikh, et al. 2010). Different granulation processes exist, and they can be approximately classified into two main groups: dry granulation and wet granulation techniques. (Oscar-Rupert Arndt et al. 2018).

However, folic acid was determined to be degraded very readily, and it is susceptible to various environmental factors such as heat, UV light, and oxygen. People who are more exposed to UV light are more likely to have a degradation of folic acid in their blood. (Morten Kristian et al. 2005).

1.3 Anemia

Folate deficiency can cause many undesired health problems. However, a critical weakness is only seen months after the reduction of the dietary in taking when the folate storage is exhausted. Frequent unwanted health problems due to folate lack are macrocytic anemia, weakness, and confusion, memory deficits, shortness of breath, peripheral neuropathy, pregnancy complications, and depression. (Siaw-Cheok et al. 2016).

Furthermore, folic acid and vitamin B12 deficiency is a compounding factor in the metabolic etiology of anemia in pediatric. (Krishna et al. .2014). Also, (Fatima Zeeshan et al. .2017) found that there was a relationship between folic acid deficiency and anemia in children.

Anemia is one of the most global public health problems affecting both developing and developed countries with significant consequences for human health as well as social and economic development. It occurs at all stages of the life cycle but is more prevalent in pregnant women and young children. (World Health Organization 2002). Globally 1.62 billion people are anemic. In India, around 89 million children are anemic. The occurrence of anemia was 70% in children aged 6–59 months. (M Tawfique et al. 2017).

In Pakistan, 61% of children under five are anemic, (Fatima Zeeshan1 et al. 2017) studied the interconnection between hemoglobin, ferritin, vitamin B12, and folic acid for 180 Pakistani children anemic under two years, 60% had low folic acid, and 4% had little in the ferritin level, and 45% had low in vitamin B12. (Fatima Zeeshan1 et al. .2017).

(Krishna Kishore Sukla et al.,2013) studied 1290 individuals have a leak in Homocysteine, related to a deficiency of vitamin-B12 and folate levels, they found in microcytic anemia 46% were vitamin-B12 deficient, 8% folic acid deficiency, while in the case of normocytic anemia it was 77%, 23% respectively. In the case of macrocytic anemia, it was 82%, 29%, respectively, at the end of the anemia diseases not only related to iron deficiency but it related to leaking in B9, B12.

On the other hand, (Tivendra Kumar et al., 2016), in north India, one thousand children under three years had anemia. They were enrolled and supplemented with placebo, folic acid, vitamin B12, or both for six months. To see the effectiveness of folic acid & B12 to improve Hb concentration. After six months for the one thousand children, the hemoglobin concentration did not develop that supported with the supplementation.

Locally, the various districts of West Bank examined children at the age of 12 months, and the percentage was 38.7% in 2017, where the rate of mild anemia was 94.2% of among anemia children, for moderate anemia was 5.8% and for severe anemia was 0.05%. (Ministry of Health, State of Palestine, 2017).

(Rima Rafiq El Kishawi 2015) studied 357 children under five years in Gaza Strip, the study aimed to decide the factors that have increased the prevalence of anemia were

income, and malnutrition were the significant factors to prevalence anemia the results showed the family has a low income and the underweight children more anemic than who had a high salary. In a healthy weight, the results as the following: 9.9 % had moderate anemia, 46.5% had mild anemia, and 40.3 % had normal hemoglobin.

Anemia is defined as a low blood hemoglobin concentration. There are many causes of anemia. Firstly, nutritional reasons, for example, iron, folate, vitamin B12, and protein deficiencies. Secondly, Non-nutritional causes, like creation factors and parasitic diseases. (Olivares M, et al. 1999).
There are various types and classifications of anemia. The occurrence of anemia is because of the different red cell defects such as production defect (aplastic anemia), maturation defect (megaloblastic anemia), defects in hemoglobin synthesis (iron deficiency anemia), genetic abnormalities of hemoglobin maturation (thalassemia) or because of the combination of abnormal hemoglobin (hemoglobinopathies, sickle cell anemia, and thalassemia) and physical failure of red cells (hemolytic anemias). (Soundarya N et al. 2018).
The most basic symptom of anemia is weakness. A low red blood cell count can also cause briefness of breath, dizziness, headache, coldness in your hands or feet, pale skin, gums, and nail beds, as well as chest pain. A severe response to a blood transfusion. Treatments for hemolytic anemia involve blood transfusions, medicines, plasmapheresis, surgery, blood, and marrow stem cell transplants and lifestyle modifications. (Soundarya N et al. 2018).

This research aims to develop a new dosage form for the folic acid target pediatric to treat the deficiency of folic acid. This research will focus on studying the physical and chemical stability under different parameters for a new solid dosage form of folic acid as granules and the difference between the techniques in preparation for these granules.

To provide a once-daily dose to children from age 0 to 4 years. This formula has three main advantages first excellent stability of solid dosage form, second excellent flexibility of liquid dosage form, and, most importantly, safe to use.

1.4 Problem Statement:

1. No report was found on the developed formulation of folic acid granules ina sachet.
2. There is a need to study the effect of pH and UV fora certain folic acid new formula.
3. More work needed for preparation folic acid alone for infants without other drugs included.
4. The liquid dosage form of folic is unstable.
5. The stability of folic acid reduces when folic acid with other vitamins.

1.5 Specific Objectives:

The aims of this research are:

1. Provide a once-daily dose of folic acid for Infant from 0 to 4 years as granules to achieve a more stable solid dosage form for folic acid and had cheap, easy, and appropriate doses for infants worldwide. The formula can be used by dissolving 0.5 g of the formulation of folic acid in 20 ml of water.

2. Prepare a new formula of folic acid that has the advantage of solid dose form with excellent stability, and still has the strength and flexibility of liquid dose forms.

3. To use two different techniques, wet and dry granulation, to prepare folic acid to reach the ideal formulation and study the certain formula of folic acid under several parameters (pH& UV).

Chapter Two

Literature review:

This chapter gives an outline of the research of dosage form and the impact of UV& pH at the stability of folic acid.

2.1 Stability of the dosage form:

(Vignesh et al., 2012) formulated liquid dosage form for oral delivery for folic acid alone. Different liquid solutions were used like purified water, sorbitol, glycerin, propylene glycol, and sugar. To determine the performance for each formula, HPLC was used and ICH criteria for the accelerated study.

The solutions of folic acid were prepared at different pH (5.0-5.5). They found after 30 days, the solution of folic acid dissolved in water was 8.86%, and the pH increased to 8.48, while the concentration of the solution of folic acid dissolved in sugar was 9.78%, and the pH decreased to 4.06.

On the other hand, when sorbitol, glycerin, and propylene glycol were used, the concentration reached 93.09 %, 92.03%, 94.08%, and the pH stayed on the range 5.0-5.5.

However, when all solution (sorbitol, glycerin, and propylene glycol) were mixed in one formula and compared this formula with three commercials, multivitamins used normal condition 25°C±2°C /60%RH±5 %.

After two years, the new formula was more stable than other multivitamins products, 82.75 %, 51.25 %,47.95% 38.45 %, respectively. Furthermore, in the accelerated study, the new formula reached 85.61 after six months. The folic acid in different solutions did not reach a stable liquid formula for use.

(Gobi et al., 2015) studied the physicochemical (pH, odor, general appearance) and microbiological stability of an extemporaneous oral suspension 1mg/ml of folic acid. The authors studied the effect of temperature in the stability of extemporaneously of folic acid stored at 4°C (refrigerated) and 25°C (room temperature). They found there was no significant change in pH, odor, general appearance, and microbiological stability after 60 days in both temperatures. There was no HPLC data to ensure the efficiency of this suspension.

On the other hand, (Awab et al., 2016). studied the chemical stability, microbiological stability, and physical factors (appearance, hardness, friability, water content, dissolution, and disintegration) of 5.0 mg tablet of folic acid, according to USP criteria. The folic acid was studied under long-term testing used 30˚C and 40˚C temperatures. The results showed the concentration of folic acid at the beginning (101.9) at 30˚C, and 40˚C after 36 months, the concentration reached to (97.0,96.6), respectively. There was no significant change in the concentration of folic acid. Furthermore, for physical factors, no significant

change occurred, and the microbiological test within limits. Also, (Larry et al., 2008) found the folic acid was highly stable in the solid dosage form.
(Omar Sarheed et al.,2014). Studied the folic acid supplements by using dissolution profile test. They compared the content of folic acid between multivitamins product content folic acid and other vitamin and tablets only content folic acid, by using USP dissolution criteria, water, and citrate buffer (pH=6.0) used as a dissolution medium. Both products passed the dissolution test of releasing the expected amount of drug substance (75%) within 60 minutes. The utilize of citrate buffer showed an enhanced dissolution behavior of folic acid tablets compared to water, but there was no significant effect on the multivitamins product. (Omar Sarheed et al., 2014).
Moreover, (Ljiljana Krsteska et al.,2011) studied the release of folic acid film-coated tablets. To find the optimal formulation of folic acid by using the dissolution test. The authors formulated five different formulations by using fillers and excipients used two techniques of granulations wet and dry. Measure the performance of the dissolution and disintegration test under the criteria of USP. The results showed the combination of hydrophilic and hydrophobic filler and suitable wetting agent in the same formula by using wet granulation technique achieved to maximum dissolution rate of 97.5%.

2.2 pH and UV impact:

(Liang et al., 2013) studied the stability of synthetic folic acid under several parameters involving pH and light. The study included two parts. The first one examined the relationship between pH and time; the second part, the relationship between pH & light at room temperature. The results showed excellent stability of the solution of folic acid at pH 8.05-10.40 after 72.0 hours but showed the stability decreased when the pH became close to 1.0. In the second part, two types of illumination (sunlight and the GZX-250 intelligent illumination incubator) were used. The results showed stability of the solution of folic acid exposed to sunlight after two hours decreased, but the solution exposed to GZX-250 still stable.

(S. Yakubu et al., 2010) investigated the degradation of folic acid. It was treated by acids, alkalis, and ultraviolet radiation. Folic acid solutions were prepared to coordinate different pH (4.2, 5.4, 6.4, 8.2) by using 0.1 N HCl for acidic medium and 0.1 N NaOH for alkali. Also, folic acid was exposed for 5 hours by UV light. They found that the folic acid solution at (pH 4.2) at acidic medium was highly degraded than at alkali and UV radiation.

(Morten Kristian et al., 2005) studied folic acid degradation in aqueous solution in aerobic conditions at room temperature after exposure to UV 365 nm irradiation at PH 7 for 20 min and 60 min and found that degradation reach to 20% and 80%, respectively. They also found that folic acid cleaved to p-amino benzoyl-L-glutamic acid and 6-formyl pterin. When the irradiation continues, 6-formyl pterin was degraded to pterin-6-carboxylic acid. They found that folic acid-sensitive to UV & the photodegradation products were to p-amino benzoyl-L-glutamic acid and 6-formyl pterin.

(Jamil Akhtar et al., 2003) Studied folic acid photodegradation products. They used TLC, HPLC, and Spectrophotometric methods to classify the products. Folic acid prepared in aqueous solution at different pH 2-10 under aerobic requirements and exposed for 10 hours by using Philips 30 WT UV lamp 254 nm. They found folic acid degraded to pterin-6-carboxylic acid and p-amino benzoyl-L-glutamic.

(Jamil Akhtar et al.,1999) investigated the photodegradation for an aqueous solution of folic acid at different pH (2.0-10.0) by exposing them to ultraviolet radiation. They found that folic acid degraded with time at different pH levels, but the reaction rate in acidic medium (pH 2.0–4.0) was higher than in basic medium (pH 8.0-10.0). The photolysis reaction increases due to the activation protonated in acidic medium was higher than the basic medium after 3-8 hours. Results showed the photodegradation of folic acid fragment were pterine-6-carboxylic acid and p-amino benzoyl-L-glutamic acid.

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Chapter Three
Materials and methods

Methodology:

This study started in April 2019 up to February 2020. Depending on the goals of this research, two types of studies were performed:

1) Those necessary to characterize the pharmaceutical quality of the manufactured folic acid products.

2) Studies needed to identify the effect of pH and UV light on the folic acid product.

To test the quality of the pharmaceutical manufacturing of folic acid products, the following tests were performed:

- Stability study:

1. Stress condition.
2. Physical appearance.
3. Disintegration.
4. Water content.
5. Weight variation.
6. Drug content determination.

Studies needed to identify the effect of pH and UV light on the folic acid product. The following tests were performed:

- Photostability.
- pH effect.

The experiment designed to evaluate folic acid under different environmental effects and to measure the sensitivity to light and pH.

3.1 Materials:

The following list includes the materials required for formulation and packaging the drug product:

Table 3. 1: Materials

N	Item	Description	Function	Assay
1	Folic acid	A yellow crystalline powder from Hebei Jiheng group.	Active ingredient	91.1%
2	Mannitol	White powder from Roquette.	Diluent	98.8%
3	Hydroxypropyl methylcellulose	White powder from Orison chemical limited.	Binder	100%
4	Cellulose microcrystalline	White granules from JRS Pharma.	Binder	100%
5	Sucrose	White beet crystal.	Sweeting agent	100%
6	Magnesium stearate	Fine, light white powder from Magnesiamineral compounds.	Lubricant	100%
7	Strawberry	Fine powder very slightly yellow from Givaudan Company.	Flavor	100%
8	Water	Water for Injection (sterile water)	Processing agent	-
9	Empty Capsule shells	Size (0) & (00)	Container	-
10	Methanol	From Merck KGaa		99.9%
11	Ammonium hydroxide	From Merck KGaa	Buffer	26.0%
12	HCl	From Merck KGaa	Adjust pH	37.4%
13	NaOH	-	Adjust pH	-

3.2 Equipments and tools:

Table 3. 2:Analytical Equipments

N.	Item	Specifications
1	HPLC Dionex ultimate 3000	UV Detector
2	Filtration system	
3	Filter membranes 0.45 micrometer	
4	Volumetric flasks	100 ml,1000 ml
5	Sonicator	
6	Analytical balances	
7	pH meter	
8	UV-Visible spectroscopy Shimadzu	
9	Incubator	30±2 C/65±5%RH 40±2 C/75±5%RH
10	UV Darkroom 365/254nm | CN-15.LC | 230V	Wavelength 365 nm

3.3 Formulation

Table 3. 3: Formulation

Formulas	B1 Wet	B2 Dry	B3 Dry
Excipient	500.0mg	500.0mg	500.0mg
Folic acid	0.33 mg	0.33 mg	0.33 mg
Mannitol	488.00 mg	-	473.00 mg
Hpmc*	10.00 mg	-	-
Magnesium stearate	1.00 mg	1.00 mg	1.00 mg
Sucrose	-	467.00 mg	-
Avicel (101) **	-	30.20 mg	25.20 mg
Strawberry	0.50 mg	0.50 mg	0.50 mg

*****Hydroxypropyl methylcellulose
**Cellulose microcrystalline

3.4 Manufacturing Procedure:

The granulation processes were used to provide the homogenize granules.

Types of granulation:

Wet granulation includes the massing of a mix of dry primary powder particles using a granulating fluid. A solvent of the granulating fluid must be volatile, can be removed by drying, and is non-toxic. (Dilip M. Parikh et al. 2010).
Usually, suitable liquids include water, ethanol, and isopropanol. (Dilip M. Parikh et al. 2010).
Dry granulation: is the dry method of granulation, the primary powder particles are aggregated at high pressure. (Dilip M. Parikh et al. 2010).

Figure 3. 1: Silverson mixer.

Figure 3. 2: Planetary mixer.

Wet granulation process:

Dissolve 10.0 g of Hydroxypropyl methylcellulose in 100.0 g water mix well in the beaker by using a Silverson mixer. Add 3.3 g of folic acid to solution make sure the solution is homogenized. Mix 90.0 g of Hydroxypropyl methylcellulose with mannitol and use sieve number 30. Add strawberry flavor to the solution, put the materials in a planetary mixer, and add the solution slowly and keep it for 15 min. Put the wet granules in the oven for 2 hours after that add 10.0 g of magnesium stearate and use sieve number 24.
Finally, use a planetary mixer for 5 min and store the formula in a dark container.

Dry granulation process with sugar:

Mix 3.30 g of folic acid with 302.0 g of Avicel and 4.67 kg of Sucrose manually, then use Sieve 30 for the previous mixture, and add5.0 g of strawberry, after that utilize planetary mixer for 15 min, add10.0 g of magnesium stearate and use Sieve 24.Mixed for 10 min by planetary mixer, then make moderate compression through JCMCO, Slagged the tablets to granules by FIT-ZIMLL, and store the formula in a dark container.

Dry granulation process sugar-free:

Mix 3.30 g of folic acid with 252.0 g of Avicel and 4.73 kg of mannitol manually through Sieve 30 for the previous mixture and add 5.0 g of strawberry for 15 min by using planetary mixer mix well then add10.0 g of magnesium stearate through Sieve 24, and store the formula in a dark container.

3.5 Evaluation:

3.5.1 Evaluation of granules:

According to ICH, evaluate the formulated granules to measure the stability at different storage conditions, and at different periods.
The granules were evaluated every three months for the following tests:

Table 3. 4: Tests

N	TEST	METHOD
1	Physical appearance	Visual
2	Water content	According to USP
3	Disintegration time	According to USP
4	Weight variation	According to USP
5	Drug content/assay	According to USP

3.6 Test methods:

3.6.1 Stress testing:

1. Incubator (25°C ± 2°C/and 60%RH ± 5%).
2. Incubator (30°C ± 2°C and 65% RH ± 5%).
3. Incubator (40°C ± 2°C/and 75% RH ± 5%).

3.6.2 Physical appearance:

The formulated granules performed visually, check the color, and any change in the shape.

3.6.3 Water content (Karl Fischer method):

The water content measure, according to a previously described method for USP⟨921⟩ Water Determination.

3.6.4 Disintegration time:

Disintegration time it is the time taken for the entire capsules to disintegrate in the medium used constant temperature and speed. The time taken by the capsules to disintegrate completely and go into fragments shall be observed. The medium to be used is water or 0.001N HCl. The temperature shall be kept constant at 37C ±0.5 C.

3.6.5 Drug content:

To measure the content of dosage units, two methods were used of weight variation or content uniformity. A content uniformity test is performed to confirm that the folic acid-containing dosage unit does not have less or more than the specific percent of folic acid relative to the label claim.

3.6.5.1 Weight variation:

For this test, 20 capsules are selected randomly from the batch, and the individual weight of each capsule is noted.

3.6.5.2 Drug content determination:

Table 3. 5:Experimental conditions for chromatographic experiments.

N	Parameter	Description
1	Mobile phase	100.0% of preparation mobile phase*
2	Internal standard	Folic acid RS
3	Chromatographic conditions	Room temperature
4	Detector	UV spectrophotometer at 280 nm.
5	Column	Li Chrospher 60RP-select B (5μm) 125*4mm.
6	Flow rate	1.5 ml/min
7	Injection volume	20 μl

35.1 g of sodium perchlorate, 1.4 g of monobasic potassium phosphate dissolves in one-liter volumetric flask add 7 ml of 1 N Potassium hydroxide and 40 ml methanol dilute with water to volume and mix. Adjust with 1 N potassium hydroxide or phosphoric acid to a pH of 7.2 and filter through 0.45 μm membrane.

3.6.5.3 Preparation of the standard:

Weigh 0.72 mg of folic acid working standard, transfer into a 100ml volumetric flask, add the diluent to volume, sonicate and mix to dissolve. Take 5.0 ml of the previous solution and dilute to 100.0 ml with the diluent and mix. After that filter, the solution by using a 0.45 mm membrane.

3.6.5.4 Preparation of the samples:

Weigh 1.0 g of formulation folic acid equivalent 0.72 mg pure folic acid, transfer into a 100ml volumetric flask, then add the diluent to volume sonicate and mix to dissolve, after that filter the sample through 0.45 mm membrane. Then by using HPLC, measure the assay.

Analysis: 3 samples prepared, and each sample replicated three times in HPLC to guarantee the accuracy of the analysis.

3.7 Studies to identify the effect of pH and UV light on the folic acid product.

3.7.1 Preparation of sample to study pH and UV effects:

1-Pure folic acid:

Dissolve 6.60 mg of pure folic acid in 100.0 ml of 0.1 N NaOH, adjust pH using HCl to reach 1.47,5.50,8.36,10.60, respectively, at room temperature After finishing the adjust ,separate the samples for three groups, first stored at darkroom, the second exposed to365NM UV chamber for one hour, and the third exposed to365NM UV chamber for two hours. The samples measured by using a UV visible spectrophotometer at λ= 265,280 nm.

2- Formulation of folic acid:

Dissolve 10.0 mg of a formula of folic acid in 100.0 ml of 0.1 N NaOH, adjust pH with HCl to reach 1.47,5.50,8.36,10.60 respectively at room temperature. Filter the solution with filter paper. After finishing the adjust, separate the samples for three groups, first stored at the darkroom, the second exposed to365NM UV chamber for one hour, and the third exposed to365NM UV chamber for two hours. The samples measured by using a UV visible spectrophotometer at λ= 265,280 nm.

Analysis: 3 samples prepared, and each sample replicated three times in a UV visible spectrophotometer to guarantee the accuracy of the analysis.

Chapter Four

Results & Discussion:

This chapter represents the results obtained through this investigation and introduces the required discussion for these results.

4.1 Physical appearance and water content:

There were no visible changes in the color in the formulation of folic acid throughout the study.

Figure 4. 1: Wet granulation formula B1.

Figure 4. 2:Dry granulation formula B2.

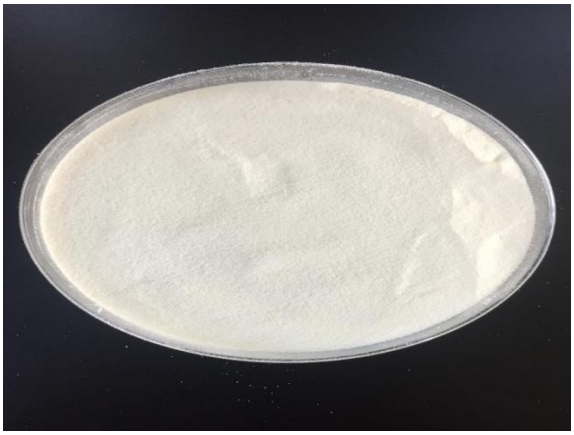

Figure 4. 3: Dry granulation formula B3.

4.1.2 Water content:

The following table showed the water content in each formula

Table 4. 1: The content of water

Formulas / Time	B1	B2	B3
0	5.00%±0.1	1.50%±0.1	1.20%±0.1
3 months	1.50%±0.1	1.30%±0.1	1.10%±0.1
6 months	0.60%±0.1	1.00%±0.1	0.80%±0.1

The relationship between time and water content in B1 showed a decrease in the content of water with the passage of time-related to participation in the reaction hydrolysis. Other formulas showed normal decreased.

4.2 Disintegration

One capsule was set in each of the six tubes of the basket, using water as a medium, and the temperature was adjusted at 37˚C ± 0.5 ˚C to measure the disintegration time. The results are shown in table 4.2

Table 4. 2:Disintegration time

Formulas / Time	B1	B2	B3
0	4.0min	2.0 min	4.0 min
3 months	4.0 min	2.2 min	4.0 min
6 months	4.2 min	2.3 min	4.1 min

There was no significant effect found in the disintegration time throughout the studyat various times.

4.3 pH

1.0 g of each formulation dissolved in 30.0 ml of tap water and using pH meter to measure the pH.The value of tap water pH was 7.1±.01

Table 4. 3: pH in tap water

Formulas / Time	B1	B2	B3
0	7.2	7.3	7.3
3 months	7.2	7.3	7.3
6 months	7.0	7.3	7.2

No significant effect found in the pH value throughout the studied at different times.

4.4 Drug content assay

4.4.1 Preparation the samples

1.0 g weighted of formulation of folic acid. Then transferred into a 100 ml volumetric flask. Added the diluent to reach to the volume, by using the sonicate mixed until all amount dissolved. Filtered through a 0.45 mm membrane. HPLC was used to analyze the samples in order to measure the performance for different formulas at different periods.

4.4.2 Wet granulation B1

The following table shows wet granulation B1 formula at different storage conditions and different periods.

Table 4. 4: Wet granulation at different conditions and various periods.

Condition	WET B 25°C± 2°C/60%±5%	WET B 30 °C± 2°C/65% RH±5%	WET B 40 °C± 2°C/75%±5%
0 time	97.84±1.5	97.84±1.5	97.84±1.5
3 Months	96.24±1.5	91.23±1.5	76±1.5
6 Months	93.66±1.5	90.16±1.5	70.46±1.5

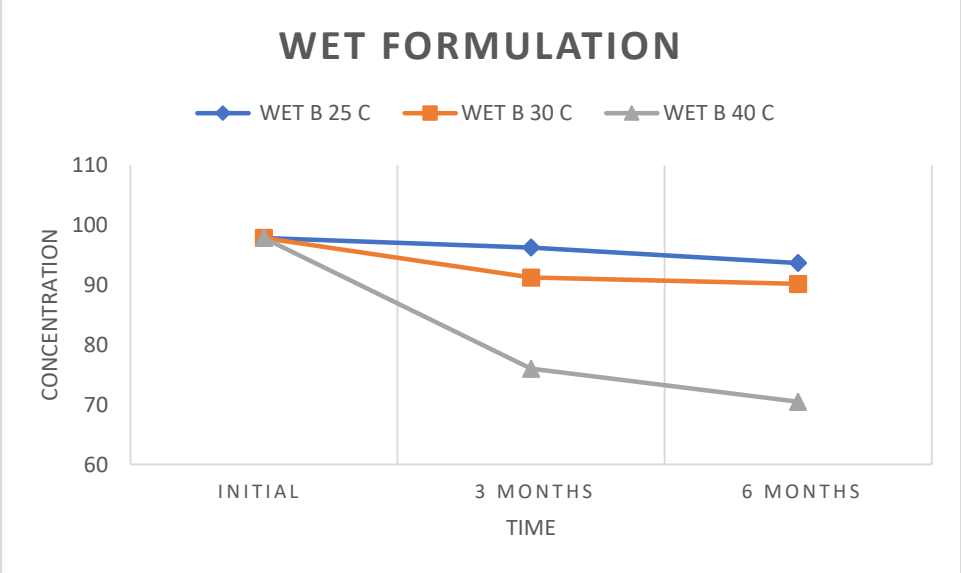

Figure 4. 4: Wet granulation performance in different storage conditions and periods.

HPLC data:

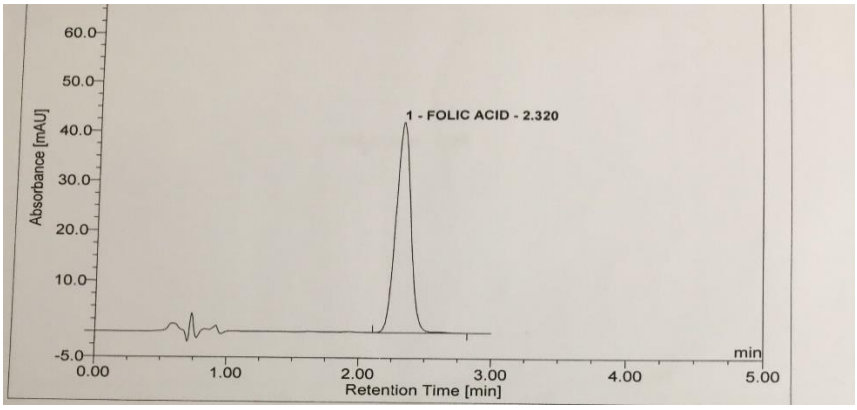

Figure 4. 5: HPLC peak for Wet granulation at time 0.

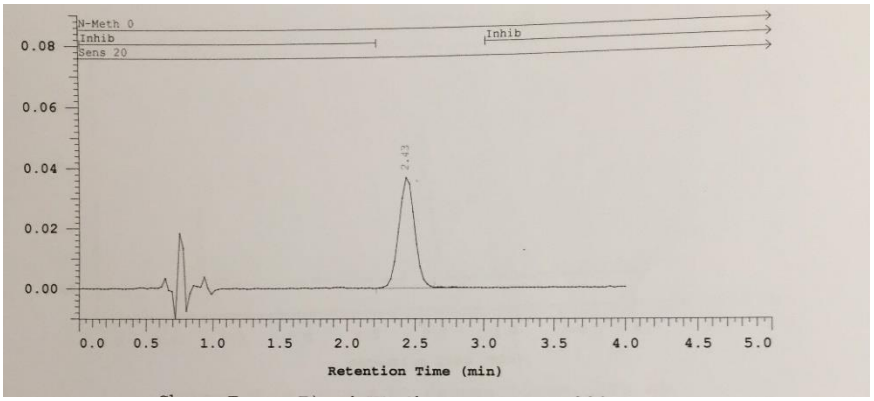

Figure 4. 6: HPLC peak for Wet granulation for three months at 25 °C ± 2°C /60% RH± 5%.

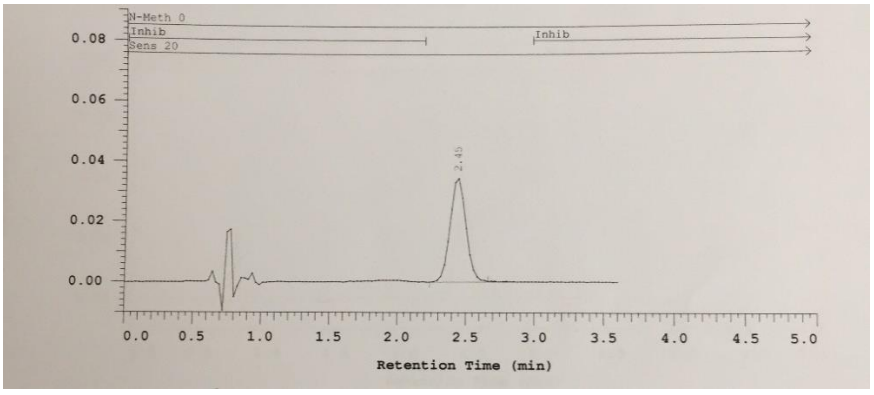

Figure 4. 7: HPLC peak for Wet granulation for three months at 30 °C ± 2°C /65% RH± 5%

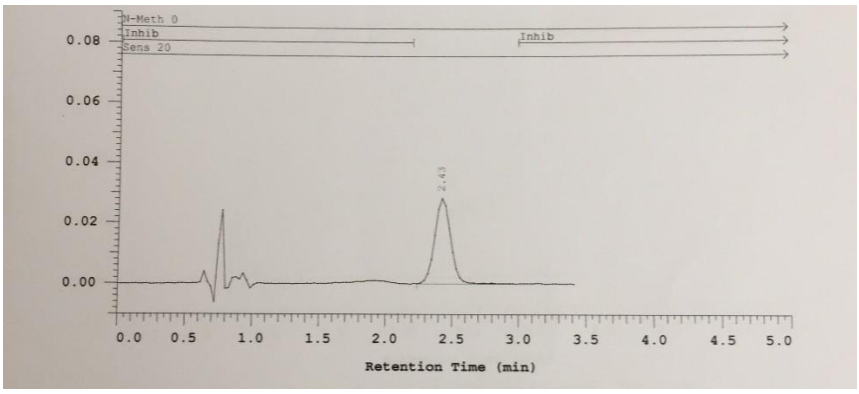

Figure 4. 8:HPLC peak for Wet granulation for three months at 40°C ± 2°C /75% RH± 5%.

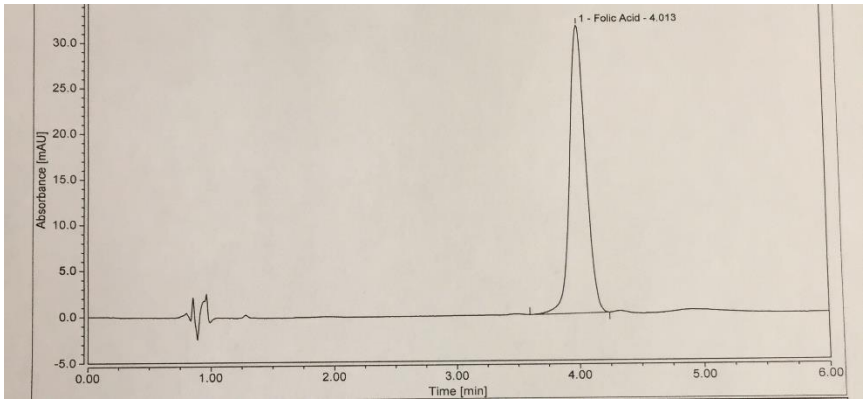

Figure 4. 9:HPLC peak for Wet granulation for six months at 25 °C ± 2°C /60% RH± 5%

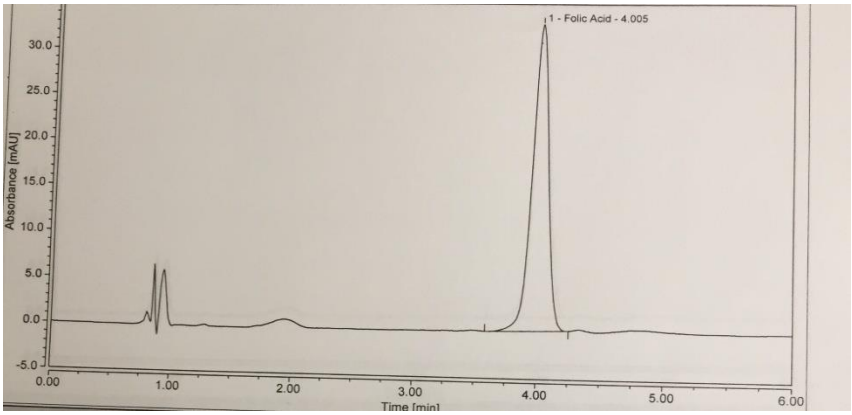

Figure 4. 10:HPLC peak for Wet granulation for six months at 30°C ± 2°C /65% RH± 5%.

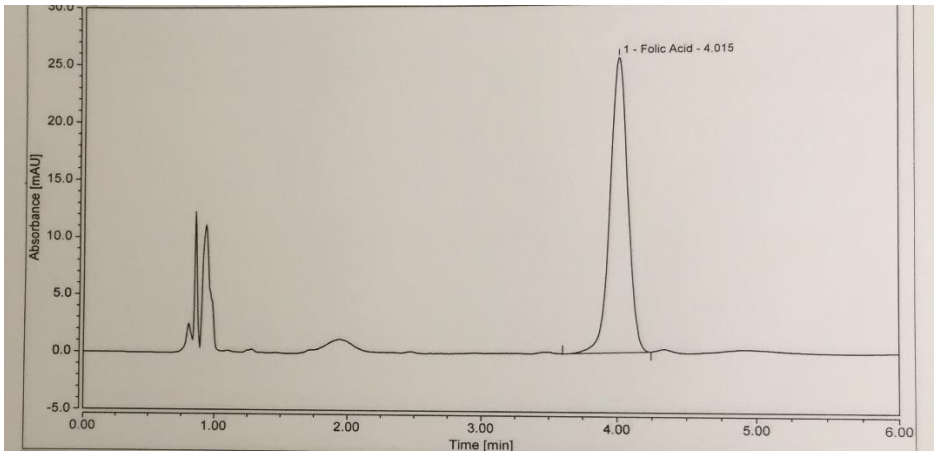

Figure 4. 11:HPLC peak for Wet granulation for six months at 40°C ± 2°C /75% RH± 5%.

Wet granulation:

The wet granulation formula reached to excellent concentration at the beginning the concentration was 97.84%, after 3 months and 6 months show excellent stability at 25 C, 30 ˚C±2˚C/65% RH±5%, respectively (96.24%,93.66%) (91.23%,90.16%). But at 40 ˚C±2˚C /75 RH±5% the concentration had a significant decrease and reached to (76.0%,70.46%) in this condition the folic acid had significant degradation, in agreement with (Menning et al. 2013) found that the impurity rate of formation in crystalline cenicriviroc mesylate tablets were manufactured by wet granulation and stored at 40 ˚C±2˚C /75 RH±5% had higher impurity than composition prepared by dry granulation. In agreement with (Sherif Badawy et al. 2004) found the tablets were prepared by using wet granulation had rapid degradation more than prepared by using dry granulation.

Furthermore (Sherif Badawy et al. 2019) found the process of wet granulation had a negative impact of some drug products on long-term stability, the loss of crystallinity associated the damaging effect of wet granulation, also (Tripetet al. .1975) found folic acid decomposed up to 1% per year in 20°C temperature and 65% RH.

The presence of water increases the rate of the chemical degradation that leading the stability for folic acid prepared by wet granulation process to degrade after three months, six months at 40 C/75% RH (76.0%, 70.46%) respectively.

4.4.3 Dry granulation:

Two various formulas prepared by using a dry granulation process. First, B2 with sugar. Second, B3 with free sugar each formula had various performance as the following figures:

4.4.3.1 Dry granulation B2

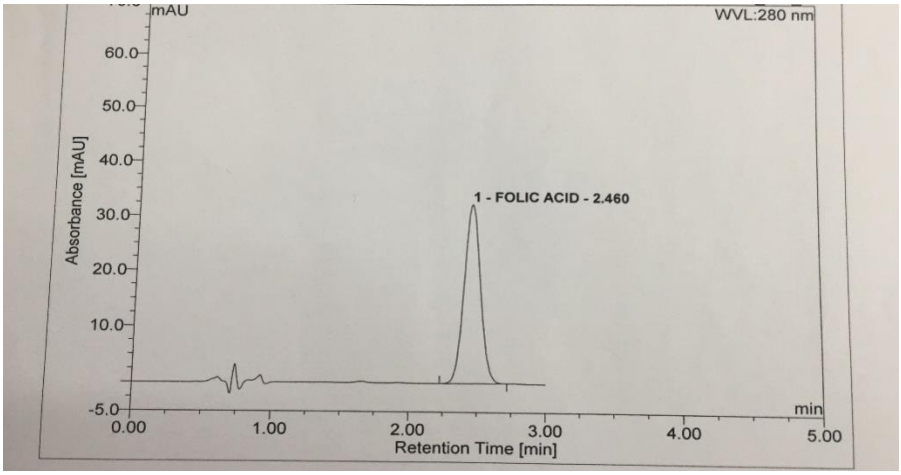

Figure 4. 12:HPLC peak for Dry granulation at 0 times for B2 formula.

The results of the B 2 formula showed problems at the mixing stage. The concentration was (80.5%) of folic acid. Due to the defect in the homogenized of the mixture, the concentration did not reach the target amount.

4.4.3.2 Dry granulation B3:

In the following table, the performance for formula B3 in various conditions and different periods.

Table 4. 5: Dry granulation B3 at the different conditions and various periods.

CONDITION	DRY B3 25 °C ± 2°C /60% RH± 5%.	DRY B3 30°C ± 2°C /65% RH± 5%.	DRY B3 40 °C ± 2°C /75% RH± 5%.
INITIAL	105.3±1.0	105.3±1.0	105.3±1.0
3 MONTHS	103.0±1.0	101.0±1.0	99.0±1.0
6 MONTHS	102.0±1.0	100.0±1.0	97.0±1.0

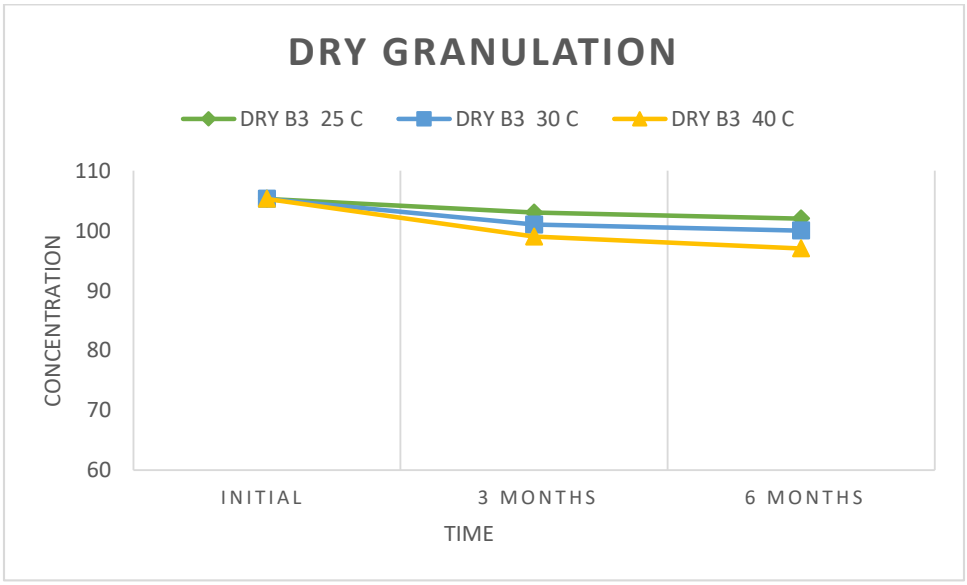

Figure 4. 13: Dry granulation B3 performance in different storage conditions and periods.

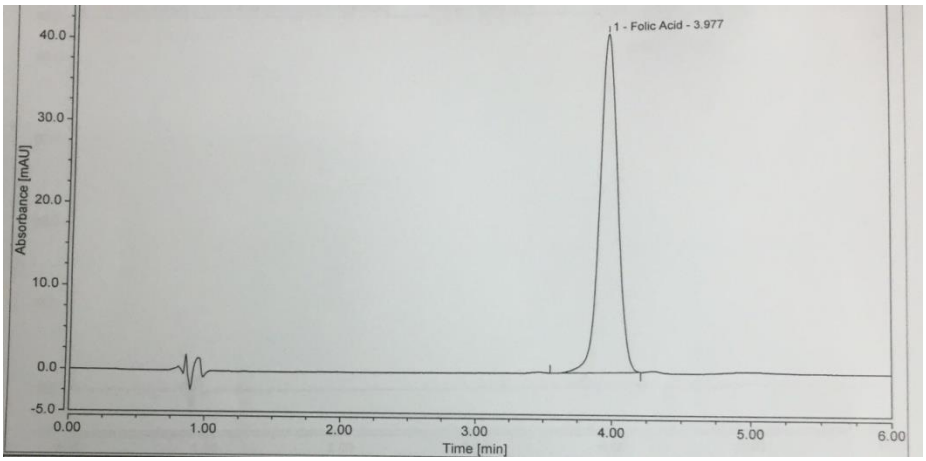

Figure 4. 14: HPLC peak for Dry granulation B3 at time 0.

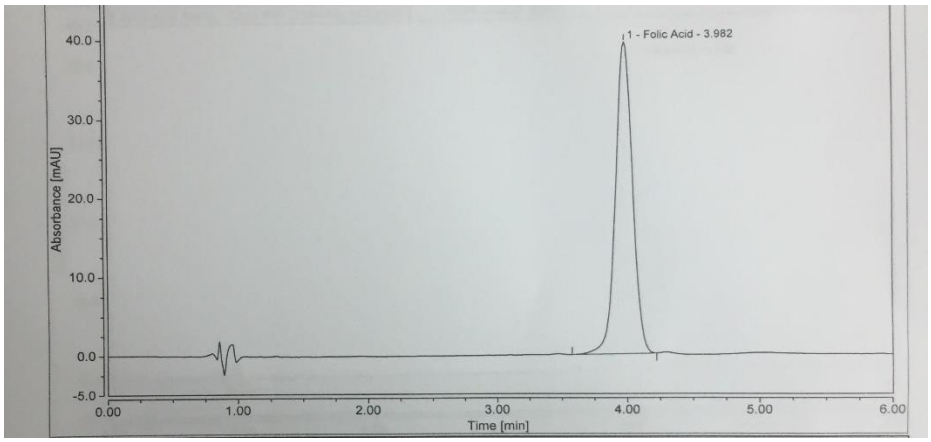

Figure 4. 15: HPLC peak for Dry granulation B3 for three months at 25°C ± 2°C /60% RH± 5%.

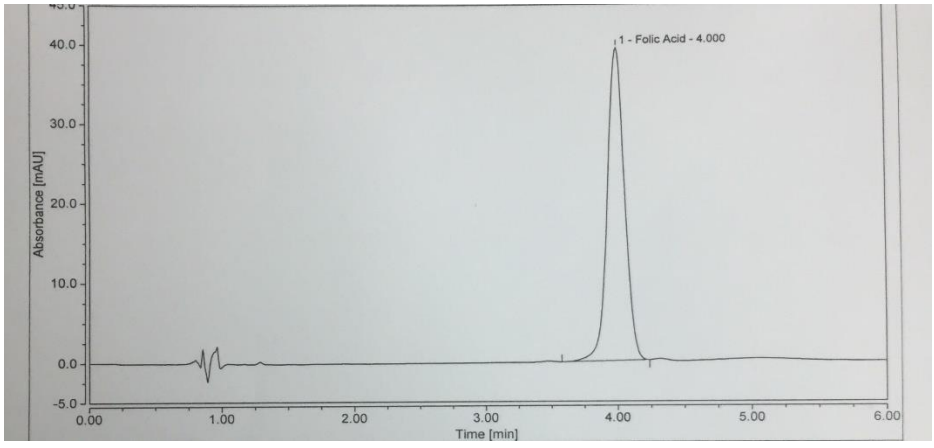

Figure 4. 16: HPLC peak for Dry granulation B3 for three months at 30°C ± 2°C / 65% RH± 5%.

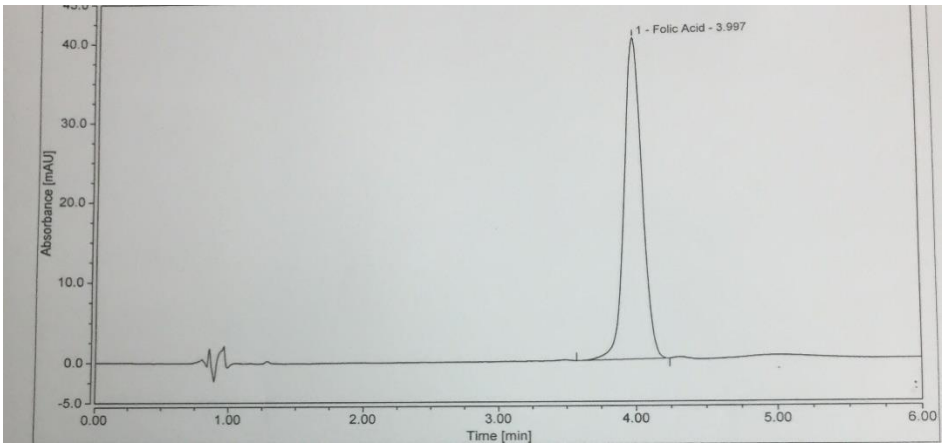

Figure 4. 17: HPLC peak for Dry granulation B3 for three months at 40°C ± 2°C /75% RH± 5%.

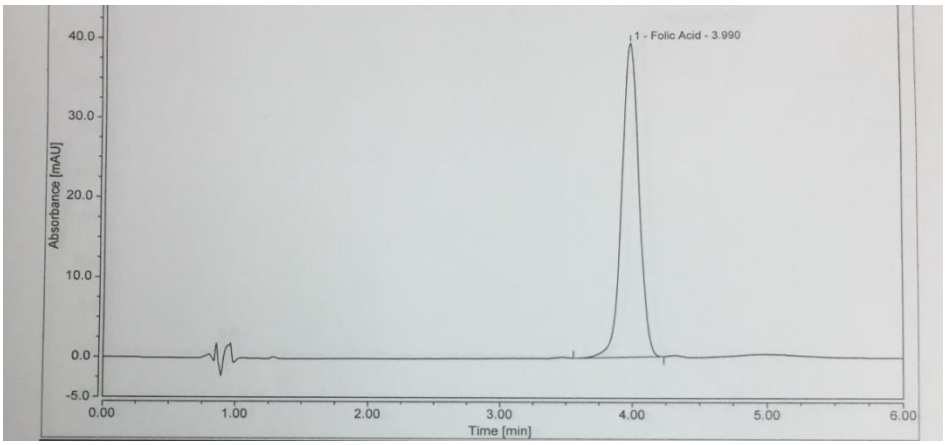

Figure 4. 18:HPLC peak for Dry granulation B3 for six months at 25°C ± 2°C /60% RH± 5%.

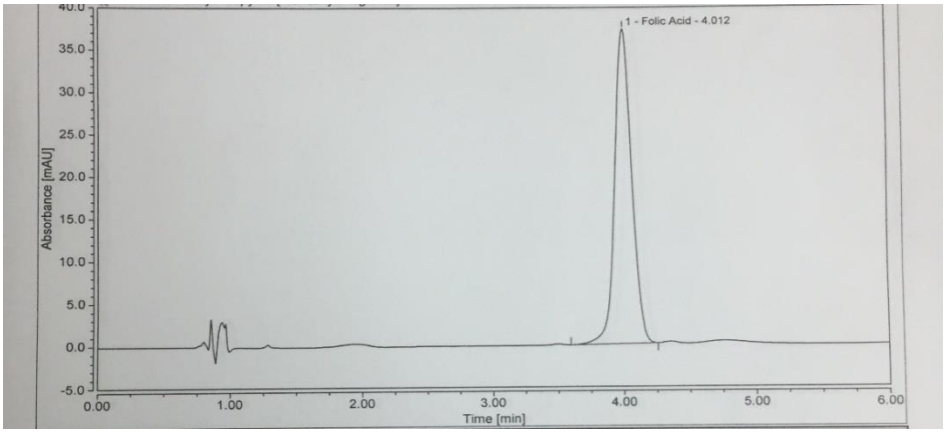

Figure 4. 19:HPLC peak for Dry granulation for six months at 30°C ± 2°C /65% RH± 5%.

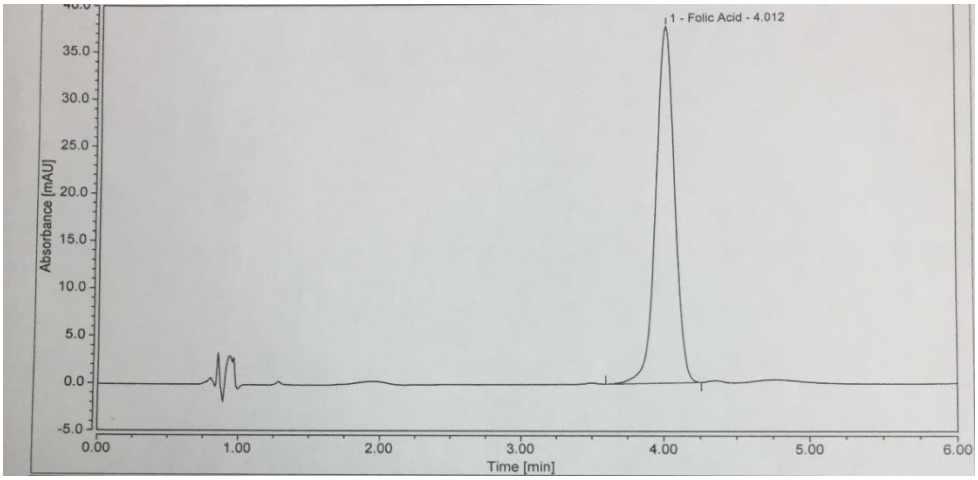

Figure 4.20:HPLC peak for Dry granulation for six months at 40°C ± 2°C /75% RH± 5%.

The dry granulation B3 showed excellent performance at stability study in several parameters and different periods at 25˚C±2˚C /60%±5 RH after six months. Only 3.3% of the concentration decreased, at 30˚C±2˚C /65%±5 RH 5.3% decreased, and at 40˚C±2˚C /75%±5 RH8.3% decreased.

This performance showed the dry granulation B3 to be the most stable formula in all periods' absence of water decrease the rate of degradation.

4.5 The effect of pH &UV on folic acid stability:

4.5.1 Preparation of the samples:

The samples were prepared at room temperature and keep in the dark area then adjust in different pH 1.47,5.50,8.36,10.36. The calibration curve with different concentration 0.66,0.033,0.0165,0.0082 mg /100 ml was investigated at these different pH. The wavelength of λ max = 265was used in order to detect the absorbance at pH 1.47.

Table 4. 6:Relationship between concentration and absorbance in the calibration curve at pH 1.47.

Concentration	Absorbance
0.0660mg/ml	1.650
0.0330 mg/ml	0.879
0.0165 mg/ml	0.470
0.0082 mg/ml	0.293
0.00 mg/ml	0.000

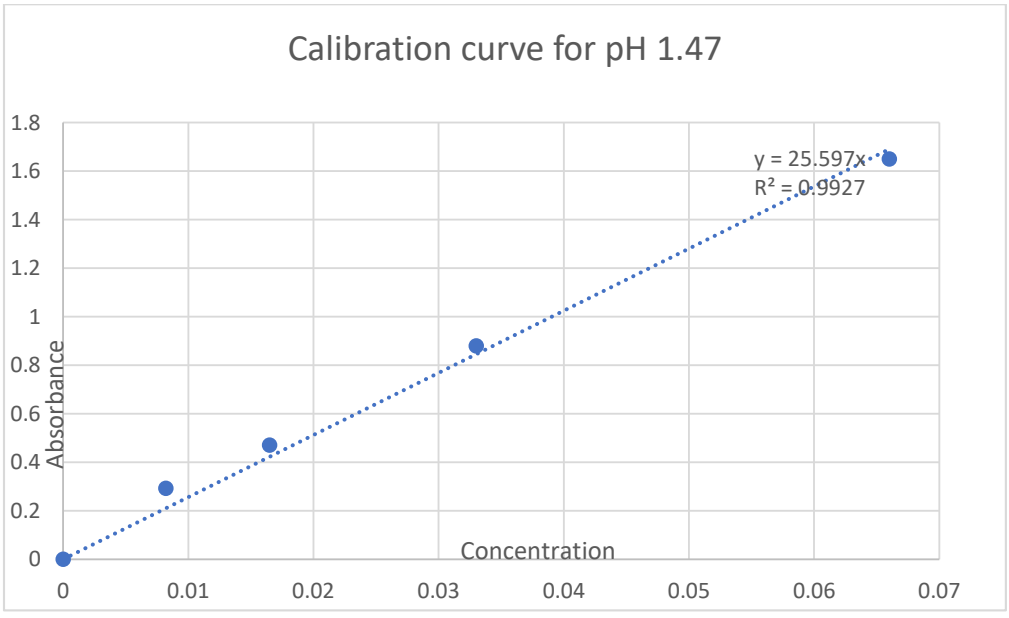

Figure 4. 21: Relation between concentration& absorbance at pH 1.47.

The wavelength of λ max = 280 nm was used in order to detect the absorbance at pH 5.50.

Table 4. 7: Relationship between concentration and absorbance in the calibration curve at pH 5.50.

Concentration	Absorbance
0.0660mg/ml	3.076
0.0330 mg/ml	1.650
0.0165 mg/ml	0.981
0.0082 mg/ml	0.533
0.00mg/ml	0.000

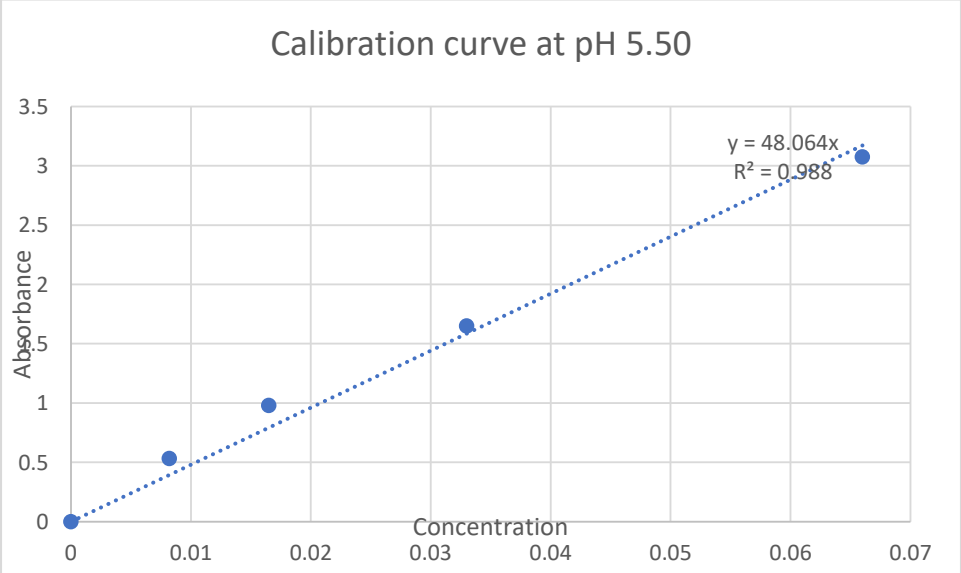

Figure 4. 22: Relation between concentration& absorbance at pH 5.50.

The wavelength of λ max = 265 nm was used in order to detect the absorbance at pH 8.36.

Table 4. 8: Relationship between concentration and absorbance in the calibration curve at pH 8.36.

Concentration	Absorbance
0.0660mg/ml	2.535
0.0330 mg/ml	1.45
0.0165 mg/ml	0.761
0.0082 mg/ml	0.343
0.0 mg/ml	0.000

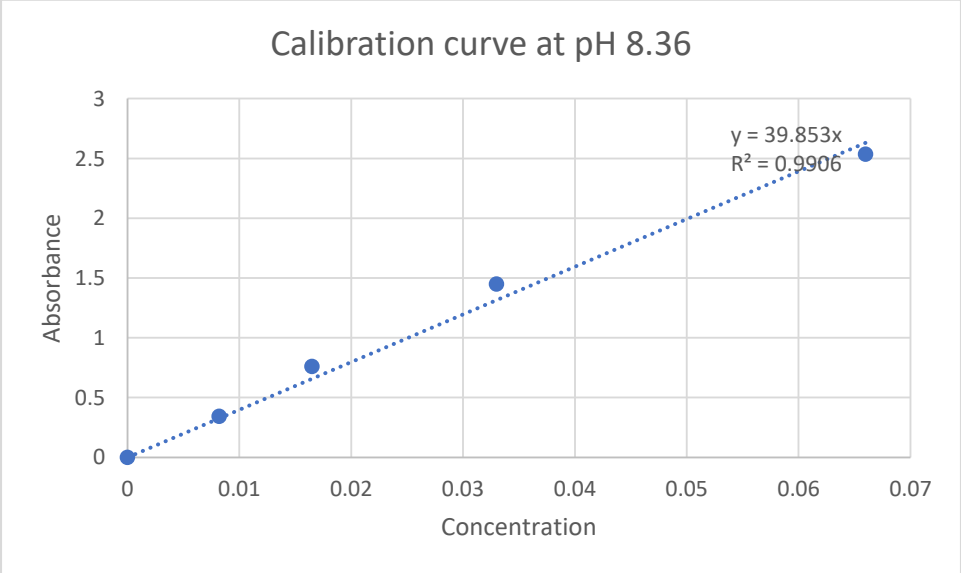

Figure 4. 23: Relation between concentration& absorbance at pH 8.36.

The wavelength of λ max = 280 nm was used to detect the absorbance at pH 10.60.

Table 4. 9: Relationship between concentration and absorbance in the calibration curve at pH 10.60.

Concentration	Absorbance
0.0660mg/ml	2.82
0.0330 mg/ml	1.462
0.0165 mg/ml	0.845
0.0082 mg/ml	0.549
0.00 mg/ml	0.000

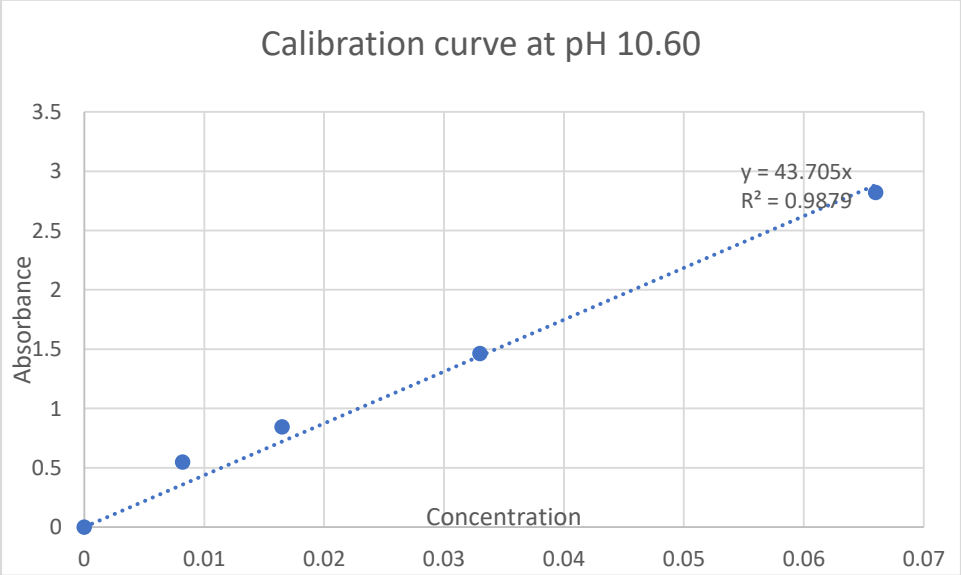

Figure 4. 24: Relation between concentration& absorbance at pH 10.60.

4.5.2 Preparation of sample to study pH and UV effects:

4.5.2.1 Pure folic acid:

The samples were prepared at room temperature and kept in the dark area before exposure to UV light then adjust in different pH 1.47,5.50,8.36,10.60 was adjusted to study the effect of pH& UV in pure folic acid. The result showed that folic acid exposed to UV light in various pH had different stability, as shown in the table.

Table 4. 10: The effect of pH and UV of pure folic acid at 365 nm

Time	pH 1.47	pH 5.50	pH 8.36	pH 10.60
0.0 H	0.06581	0.06569	0.06585	0.06576
1.0 H	0.06204	0.06062	0.06533	0.06528
2.0 H	0.05831	0.05819	0.06481	0.06470

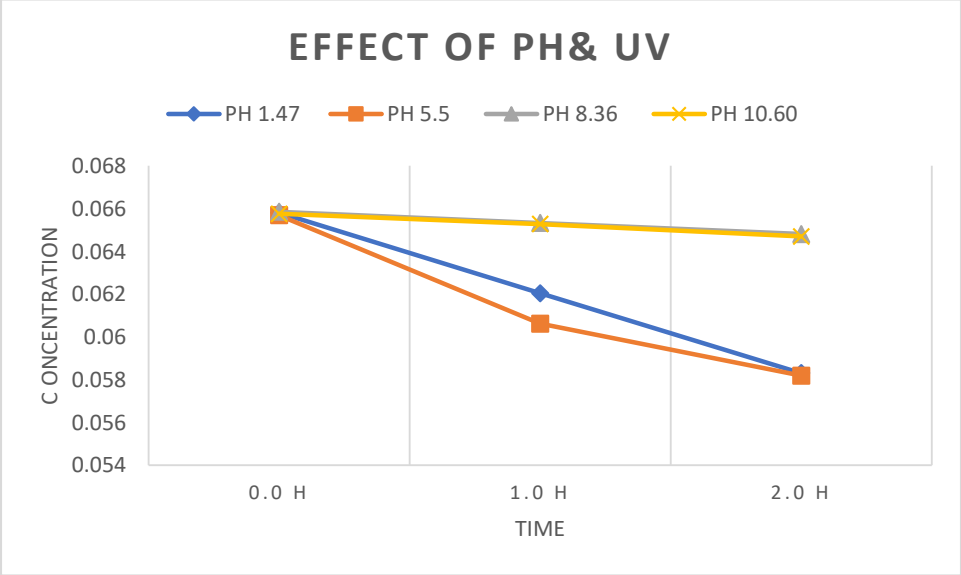

Figure 4. 25: The effect of pH& UV in pure Folic acid.

4.5.2.2 Formulation of folic acid.

The samples were prepared in room temperature and kept in the dark area before exposure to UV light, adjust to different pH 1.47,5.50,8.36,10.60was adjusted to study the effect of pH & UV in the stability of dry formulation of folic acid, The result showed folic acid exposed to UV light in various pH had different behavior as shown in the table.

Table 4. 11: Effect of pH and UV in the formulation of folic acid at 365 nm

Time	pH 1.47	pH 5.50	pH 8.36	pH 10.60
0.0 H	0.06544	0.06507	0.06567	0.06574
1.0 H	0.05651	0.06364	0.06522	0.06477
2.0 H	0.05549	0.05982	0.06335	0.06397

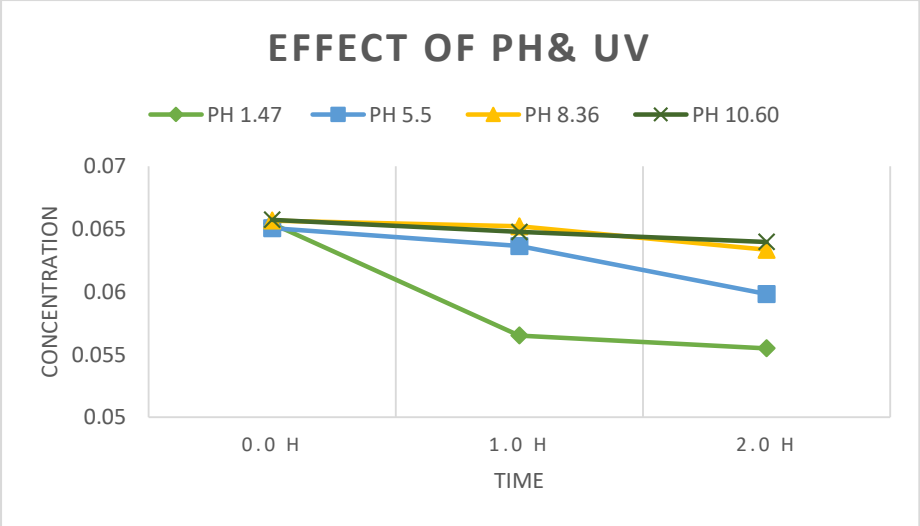

Figure 4. 26:Folic acid not stable in an acidic medium the degradation happened at pH 1.47,5.50, and more stable in a basic medium at 8.36,10.60.

The stability of folic acid in acidic medium (1.47,5.50) were less stable than the basic medium (8.36,10.6) in both pure folic acid and formulation folic acid.

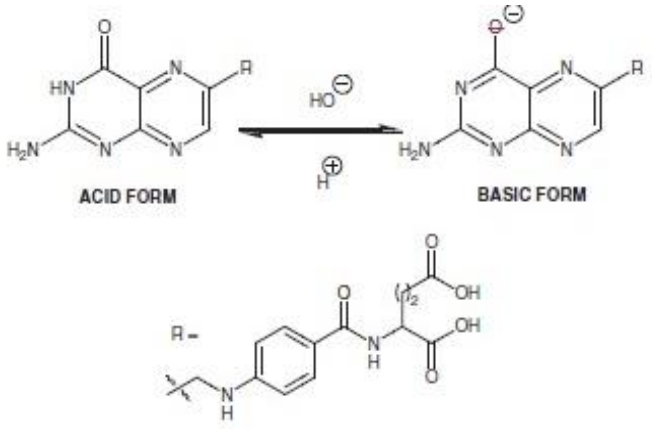

Figure 4. 27: The equilibrium of folic acid molecule between acid and basic form (Amirah et al., 2016).

The results of pure folic acid in acidic medium (1.47,5.50) showed the loss of concentration reached (12%,11.5%), respectively & for the formula of folic acid in acidic medium (1.47,5.50) showed (16%,8%), respectively. These results matched (Liang et al. 2013) studied the folic acid in acidic medium. The concentration reached less than 55% at pH (1.88,5.39,6.39), and below 15% at (2.51,3.57).

Likewise, (S.Yakubu et al. 2010) noticed the rate of degradation of folic acid in acidic medium (4.2,5.2,6.4) after exposure to UV light for 5 hours more than a basic medium (8.20). Also (Jamil Akhtar et al. 1999) studied the folic acid at pH 2.0-10.0had investigated the rate of reaction is high in an acid medium pH (2.0-4.0) because it is highly susceptible to photolysis because of the formation mostly protonated species which undergoes photolytic degradation.

The results for pure folic acid in basic medium (8.36,10,60) showed (2.5%,2.4%) respectively& for the formulation of folic acid at same conditions showed (2.5%,3.8%).
The results in agreement with (Liang et al. 2013)studied the pH(8.05,9.10,10,40) showed good stability the concentration reached up to the 93.0%, also (Jamil Akhtar et al. 1999) found the rate of reaction is low in pH (7.0-10.0) in the alkaline region because the deprotonated of the molecules happened when the pH closed to (10.0) which is much less susceptible to the photodegradation process.

The continuous exposure to UV for 2 hours in the acidic region showed photodegradation occurred during this period that matches with (Jamil Akhtar et al. 2003) found after exposure to UV at the different time two photoproducts appeared pterin-6-carboxylic acid and p-amino benzoyl-L-glutamic, in agreement with (Morten Kristian et al.2005) found

after exposed the folic acid to UV 365 nm, it is cleaved to p-amino benzoyl-L-glutamic acid and 6-formyl pterin, the continuous irradiation degraded the 6-formyl pterin to pterin-6-carboxylic acid they found folic acid-sensitive UV.

(Akhtar et al. 2003) Explained what occurred through hydrolysis of 'enamine,' the intermediate formed during photolysis, the photodegradation occurred through a free radical mechanism, that leading to the formation of p-amino benzoyl-L-glutamic acid and Pterine-6-carboxylic acid.

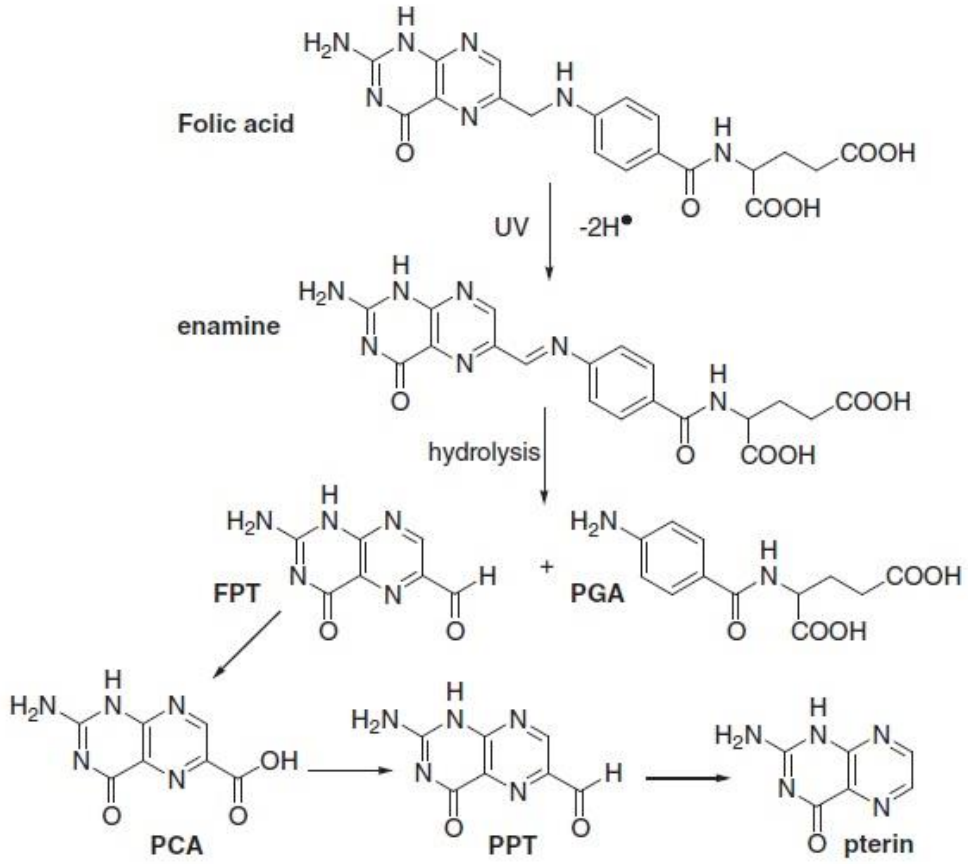

Figure 4. 28:Enamine formation and further reaction (Amirah et al.,2016).

The obtained results strongly indicate, as shown in figures (4.25,4.26), that there was an agreement to folic acid stability and pH value. When the pH decreased, the stability decreased. The exposure to light continuously increased the rate of degradation that noted after two hours. The loss of concentration reached 16% in pH 1.47.

Furthermore, when comparing the results of figures (4.25,4.26), at pH 5.50 showed enhancement in the stability for certain folic acid formula. In the pure folic acid, the loss in concentration reached 11.5%, but in a certain formula, the loss of concentration reached 8.0%. That reached a certain folic acid formula that had improved the stability of folic acid.

The conclusion of this research a certain formula of folic acid-sensitive to light, and the product can be stable for two hours after dissolves it in water.

Conclusion

Folic acid is known as vitamin B9. It can treat megaloblastic anemia. Folic acid is an important compound involved in many necessary biochemical processes in humans, mostly in its ionic form. The lack of suitable folic acid dosage form for children creates a need to cover this category, the bioavailability of folate taken from a pharmaceutical preparation is more effective than dietary folate due to the fact that 10% to 50% is lost in the cooking process. The optimum process to preparation folic acid is granulation, including dry and wet granulation, to provide a stable and homogeneous solid dosage form of folic acid. It was determined to be degraded very readily, and it is susceptible to various environmental factors such as heat, UV light, and pH.

In this work, three different formulas of folic acid were prepared as granules by using two methods dry and wet granulation. HPLC was used to determine the folic acid concentration during the stability study period, and UV visible spectrophotometer was used to measure the absorbance for folic acid after exposure to UV light at different pH. After six months of storing folic acid at several condition HPLC determined the B3 formula that prepared by dry granulation to achieve 97% under 40C/75% RH. On the other hand, B1,which was developed by wet granulation, reach 70.46% under 40C/75% RH. B3 formula was compared with pure folic acid and both exposed to UV light and prepared at different pH.

UV visible spectrophotometer showed that folic acid made at low pH (acidic medium) had high degradation than the one prepared at high pH (basic medium). The degradation rate for samples at pH (1.47) was16%, while for those at pH (10.60) was 2.5%.

The dry granulation methods are more stable than wet granulation in prepared folic acid due to the presence of water, which increases the hydrolysis. The sensitivity for folic acid to light plays as a significant factor in photodegradation, decreasing its stability. The basic medium was found to yield a more stable formulation than acidic medium when preparing folic acid as a solution.

To increase the stability of folic acid, it should be protected from light sources and kept at solid-state structures and not keep it as a solution due to increased hydrolysis.

Chapter Five

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تحضير وتقييم حمض الفوليك لأمراض الأطفال

إعداد: بهاء مجدي صبري

المشرف الأول: البروفيسور ابراهيم كيالي
المشرف الثاني: الدكتور محمود الخطيب

الملخص

حمض الفوليك هو فيتامين قابل للذوبان في الماء. وهو مركب مهم يشارك في العديد من العمليات البيوكيميائية الضرورية في البشر. يستخدم على نطاق واسع لعلاج العديد من أمراض الأطفال مثل فقر الدم وعيوب الأنبوب العصبي. نقص الجرعة المناسبة للأطفال خلق حاجة لسد احتياج هذه الفئة.
يهدف هذا البحث إلى تطوير تركيبة من حمض الفوليك تتناسب مع احتياجات الأطفال ودراسة تأثير الحرارة والضوء ودرجة الحموضة على هذه التركيبة.
في هذا العمل ، تم تحضير ثلاث صيغ مختلفة من حمض الفوليك على شكل حبيبات.تم استخدام طريقتين للتحبيب جاف والرطب.لتحديد تركيز حمض الفوليك خلال فترة دراسة الثباتية ،تم استخدام جهاز الكروماتوغرافيا السائل عالية الدقة و استخدام مقياس طيفي مرئي للأشعة فوق البنفسجية لقياس امتصاص حمض الفوليك بعد التعرض لضوء الأشعة فوق البنفسجية عند درجة حموضة مختلفة.
أظهرت النتائج أن التركيبة الرطبة وصلت إلى 97.84% من تركيز حمض الفوليك في البداية, و لكن بعد ستة شهور من دراسة الثباتية 40 درجة مئوية ±2 مئوية و 75% ±5 نسبة الرطوبة وصلت إلى 70.46%.
من ناحية أخرى, بلغت نتائج الصيغة الجافة في البداية إلى 105.3% من تركيز حمض الفوليك و بعد ستة شهور من دراسة الثباتية 40 درجة مئوية±2 و 75%±5 نسبة الرطوبة وصلت إلى 97.00%.
أظهرت نتائج دراسة تأثير الضوء و درجة الحموضة أن حمض الفوليك عند الوسط الحمضي (1.47,5.5) يتحلل , فقد تحلل ما نسبته (16%,8%) على التوالي من تركيزه بعد ساعتين من التعرض لضوء 365 نانومتر.
مع ذلك, فأن حمض الفوليك في الوسط القاعدي (8.36,10.60) له تصرف مختلف, نسبة فقدان التركيز كانت (3.5%,2.6%) بعد ساعتين من التعرض ضوء 365 نانومتر.
وجود الماء أدى إلى زيادة معدل التفاعل في طريقة التحبيب الرطب.
وجود حمض الفوليك في الوسط الحمضي زاد من انقسامه بالاضافة إلى تعرضه إلى الضوء.