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Preparation of Topical Folic Acid Gel And Investigation the Effects of Different Penetration Enhancers On The Drug Permeation Rate

تحضير حامض الفوليك على شكل جل موضعي و التحقق من تأثير محسنات النفاذية على درجة نفاذيته

By
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Palestine, June 2018

Osama Alfar

Declaration

I certify that this thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and this thesis has not been submitted for the higher degree to any other university or institute.

Signed: ……..

Osama Alfar

Date: 25/08 /2018

Table of contents

List of Abbreviations

API	Active pharmaceutical ingredient
AR	Analytical reagent
BP	British pharmacopeia
C	Concentration
C_1	Concentration in the membrane in the donor compartment
C_2	Concentration in the membrane in the receiver compartment.
C_d	Concentration in the donor compartment
C_r	Concentration in the receiver compartment
C_{sa}	Concentration of sample
D	Diffusion coefficient
ER	Enhancement ratio
FDC	Franz diffusion cell
h	Membrane thickness
HPLC	High performance liquid chromatograph
IPA	Isopropyl Alcohol
IPM	Isopropyl Myristate
K	Partition coefficient
m	The amount of material
P	Permeability coefficient

PE	Penetration enhancers
PG	Propylene glycol
RPM	Rounds per minute
r_{sa}	HPLC reading of sample
r_{st}	HPLC reading of standard
S	Surface Area
SLS	Sodium lauryl sulfate
T	Time
T_L	Lag Time
TEA	Triethanolamine
U.V.	Ultraviolet
USP	United states pharmacopeia
x	The distance in cm of movement perpendicular to the surface of the barrier
PSE-15	Polyoxypropylene Stearyl Ether

List of Tables

List of figures

Abstract

Folic acid is a B group component of vitamins and it plays role in the production and maturation of red blood cells normally. It is available in the market as tablet dosage form and in combination with iron compounds and other B vitamins. Folic acid (FA) seems to have skin regeneration properties and it can modulate DNA repair in UV-damaged skin by improving the skin elasticity and moisturizing, decreasing trans-epidermal water loss and skin roughness without any significant change of sebum secretion.

Topical FA preparations are promising due to the ease of application and acceptability by the patients, but they are not effective because they do not penetrate the skin well.

The aim of this thesis is to investigate the effects of some penetration enhancers on permeation of newly developed FA gel, as a model of semisolid topical dosage form.

In the first phase the experiment diffusion parameters of (0.1% w/w) Folic acid gel was specified and used as a reference control to measure the effects of different penetration enhancers on its permeability. In the second phase a pig skin was used to test the permeation of Folic acid from the selected gel-penetration enhancer system.

Franz diffusion cell was used in this in-vitro study. In the first phase the membrane was composed of natural eggshell membrane soaked in isopropyl Myristate. The receiver is filled with phosphate buffer pH 7.4. The donor compartment contained 5 g of gel. In the second phase, pig skin obtained from 9-weeks female pig skin was used to separate the receiver and donor compartments.

Samples of 1 ml volume were taken from the phosphate buffer at the receiver compartment after the first hour, and every hour later on up to (7) hrs for each experimental sample. FA was quantified by using HPLC at $\lambda = 254$ nm.

The solubility of folic acid was determined in acetate buffer pH 4.5 and in phosphate buffers with different pHs. The highest solubility was found in phosphate buffer pH 7.4 with a value of 0.680g/100ml.

The compatibility of folic acid was tested with different excipients (Benzyl alcohol, Carbopol, IPM, Tween 20, PSE-15, SLS, IPA, and TEA) for three days at room temperature and at 40 ºC. It was found that FA is compatible with all excipients except with Carbopol that causes precipitation.

The penetration enhancers (PE) under investigation were Benzyl alcohol, Tween 20, Sodium Lauryl Sulphate, PSE-15, IPM and IPA. They were added in different concentrations to 0.1% FA gel in the donor compartment. Diffusion parameters determined were cumulative amount, slope and intercept of cumulative amount which is plotted versus time, T_L, D, P, and K. The (ER) was used as criteria for selecting the best penetration enhancer. The enhancement ratio has been found to increase in the order of:

Benzyl alcohol (1%) > Benzyl alcohol (1%) + 20% IPA > Benzyl alcohol (1%) + 2% IPM> Benzyl alcohol (1%) + 30% IPA

FA gel containing 0.1% folic acid and the selected penetration enhancer (Benzyl alcohol) showed significant higher enhancement ratio than other agent's formulae. The final gel formula with the optimal concentration of penetration enhancer was then tested for permeation through pig skin. The API showed good penetration through the skin with a permeability coefficient of 0.005 cm/hr. The diffusion parameters for FA gel through natural eggshell membrane and pig skin are summarized in the following Table:

Comparison of Diffusion parameters for **FA gel,** containing benzyl alcohol 1% as both preservative and penetration enhancer through eggshell membrane and Pig Skin using Franz diffusion cell

	Eggshell membrane	Pig Skin
T_L [h]	1.58 h	4.78
D [cm^2. h^{-1}]	4.22 $x10^{-5}$	5.89 $x10^{-4}$
P	0.036	**0.005**
K	16.9	1.1

The stability of FA gel was tested by incubating the finished products in their final package (aluminum tubes) at different storage conditions i.e. 25 ± 2 ℃ / 60 % ± 5% RH, 30 C ± 2 ℃ / 60% ± 5% RH and 40 C ± 2 ℃ / 75% ± 5% RH. Samples were tested after two weeks and six weeks for the content of FA (assay), physical appearance, pH, and viscosity.

FA 0.1% gel proved to be stable in all aspects for the period tested (6 weeks) at all storage conditions.

Part one

Introduction

1.1 **Transdermal and Topical Delivery Systems for Drug**

Transdermal systems for drug delivery are used in order to have a continuous and controlled penetration of drugs to a localized area of skin or to the systemic circulation without passing through the stomach and avoiding the first pass hepatic effect. (Hosney , *et al*, 1998) , (Ranade , Hollinger , 2004).

The limiting step or slowest permeation in the penetration process is provided through the *stratum corneum*, or horny layer. (Evrard ,*et al*,2001) , (Elsayed ,*et al*, 2007). It consists mainly of two components that resembles a brick and mortar model in which corneocytes that form the bricks are made up of fibrous protein connections and the intercellular lipid matrix forms the mortar which contains free fatty acids, ceramides, free sterols, , cholesterol sulfate, triglycerides and sterol esters but with no presence of phospholipids. (Ramachandran, Fleisher, 2000)

In order to limit the diffusional barrier, scientists have been using some compounds for enhancing penetration called penetration enhancer (skin accelerants or promoters). (Choi, Maibach, 2005-a),(Lopez, A, *et al*,2000). Penetration enhancer may increase the drug's diffusion coefficient by deactivating, releasing, or giving rise to acyl chain defects of the *stratum corneum lipid* bilayer. Deactivation helps in the formation of micro-cavities in the bilayer which increases drug diffusion. (Ting, *et al*,2004) ,

Penetration enhancer must be pharmacologically inert and cosmetically acceptable with a specific and fast and reversible action. (Williams, Barry, 1991). Some of these enhancers may fail commercialization primarily as a result to their related mechanisms of action and potential toxicity. (Junginger, Verhoef , 1998).

1.2 **The structure of the skin**

It is well known that the largest organ of the body is the skin and it is standing for around 11% of the body mass, it is the organ that makes the body able to interact with its environment. Four layers compose the skin: the nonviable epidermis called the *stratum corneum*, the other layers are the epidermis (viable epidermis),

dermis and the one known as the subcutaneous tissues. Beside that there are several connected appendages: hair follicles sweat ducts, apocrine glands and the nails. (Walters,2002).Figures (1.1 and 1.2) illustrate the structure of the dermis and epidermis of the human skin and mammalian skin respectively.

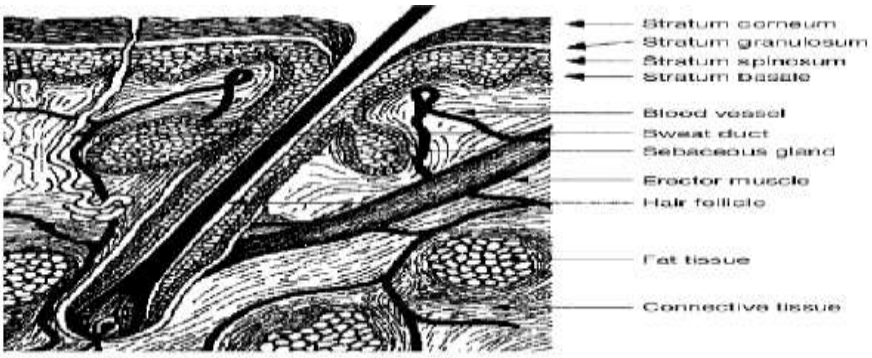


Figure1.1: Components of human skin (dermis and epidermis). (Walters,2002).

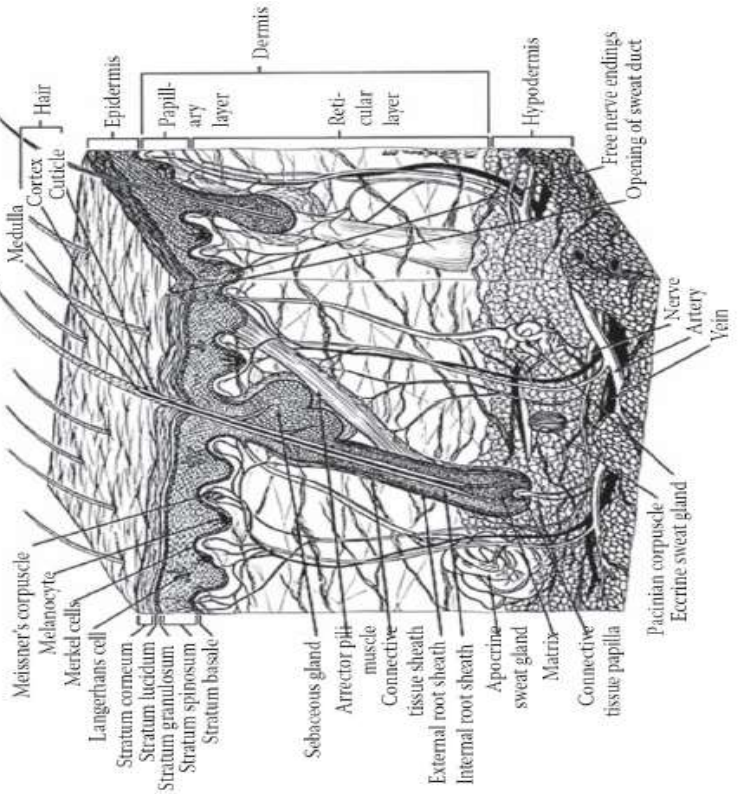


Figure 1.2: Structure of mammalian skin. (Riverie, 2006)

1.3 Function of the skin

There are many functions of the skin that allows the survival of human beings (Banker, Rhodes, 2002):

- The skin acts as a chemical barrier, preventing chemicals from penetrating the body.
- The skin is considered to be a radiation barrier, preventing UV light from penetrating the human body.
- The skin is an electrical barrier, preventing the flow of an electrical current from entering the body.
- The skin helps control body temperature and take rid of waste products.
- The skin acts as a microbial barrier, preventing microorganisms from reaching the viable tissues
- The skin regulates blood pressure and prevents dehydration by preventing the loss of life-sustaining body fluids.
- The skin by containing Merkel cells is considered as is a major sensory organ.

1.4 The Stratum Corneum

It is the external most layer of the epidermis with a thickness between 10.5 and 20 μm (15-25 cells layer thick), it is composed of very closely compact dead cells linked together in a planar arrangement with desmosomes, embedded in between is the intercellular lipid matrix. Each cell is nearly 0.5 μm thick, and 40 μm in diameter, thickness of the *stratum corneum* differs according to the part of body for example the SC in some areas like the hand palms and feet soles is thicker than SC located at other areas in the human body .Thus, in general the penetration of solutes is slower through these regions. (Walters, 2002). The corneocytes are made of fibrous protein connections and the intercellular lipid matrix is composed of ceramides, sterols, fatty acids(free),cholesterol sulfate , triglycerides, sterol esters. (Figure1.3). (Walters,2002), (Ho,2004)

There is no presence of nuclei or cytoplasmic organelles in the keratinocytes because of that the *stratum corneum* is considered to be a dead tissue. (Walters,2002)

The turnover time is the period between the production of daughter epidermal cells and their peeling from the external surface of the epidermis, it is around 28 days in normal skin and the direction of movement of these cells is toward the upper surface (Ho,2004).

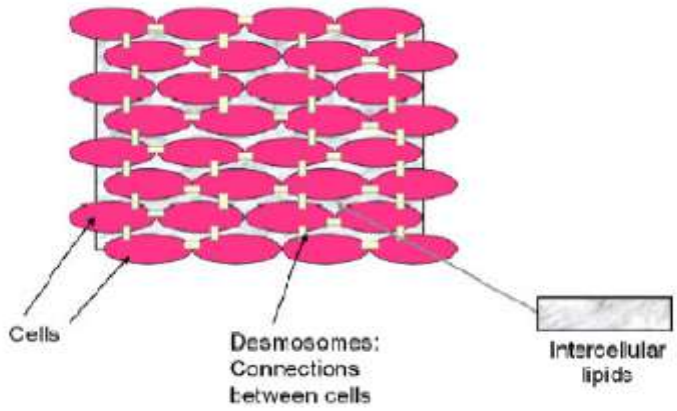


Figure 1.3: A ‘‘Bricks and Mortar’’ model for human *stratum corneum* illustrating the corneocyte ‘‘Bricks,’’ the Intercellular lipid ‘‘Mortar” and the desmosomes connecting the corneocytes. (Wickett, Vsscher , 2006)

1.5 Advantages of Transdermal Delivery System

Transdermal delivery of drugs has many advantages (Walters, 2002):

- No first-pass hepatic metabolism.
- No effects of GIT enzymes and its PH change.
- Avoidance of any GIT disturbances, such as bleeding or irritation that may be caused by some drugs.
- Avoidance of variation in the plasma concentration resulting from mixing drugs with food in the stomach.
- A more-controlled, noninvasive method of delivery, can be achieved using transdermal route with the added advantage of being able to stop absorption incase of overdose or other problems

1.6 limitations of Transdermal Delivery System

Transdermal delivery has many limitations (Walters, 2002):

- Not all compounds are suitable for this route of administration. Some topical products may give rise to some irritation and other side effects.
- Differences in permeation rates can be recorded among individuals, different races, and different ages.
- Permeation rates are influenced if the skin is diseased and how is the extent of disease.
- Sometimes bacteria that live on the skin surface can break specific types of drugs before drugs can penetrate SC.

1.7 Factors affecting functioning of skin barrier

Different factors can affect skin barrier properties, some of which are listed below:

1.7.1 Age, Gender, and Race

Premature neonates have more permeable skin, the skin of males and females may differ greatly in permeability. Also, there are great differences in skin barrier characteristics of skin across human races. The horney layers of the Caucasian skin has less cell layers with smaller density. As a result, black skin has been found to be several folds less permeable than of Caucasian skin. (Banker, Rhodes,2002)

1.7.2 Unhealthy condition of the skin

During skin diseases such as atopic dermatitis, psoriasis and contact dermatitis the composition of the lipids of the *stratum corneum* inside the intercellular lipid matrix vary from that of healthy skin. The ceramide content of the *stratum corneum* in patients with diseased skin (dermatitis) has been found to be reduced, whereas, the amount of free fatty acids in the *stratum corneum* and the ceramide profile is altered in patients with lamellar *ichthyosis* , due to these variations that

affects the barrier integrity several folds increase in permeability could be observed in diseased skin. (Choi,Maibach,2005-b)

1.7.3 Humidity may influence skin barrier

The hydration of skin increases skin permeability by changing the solubility of a permeate in the *stratum corneum* and hence can modify its partitioning from the vehicle into the membrane .In addition, hydration may cause disruption of the permeation domains possibly by swelling the polar head group regions of the lipid bi-layers. Also the corneocytes may capture water and swell. This swelling of cells may affect the intercellular lipid structure causing some perturbation to the bilayer packing. (Ting,*et al*,2004)

1.7.4 Temperature influences skin permeability

Thermal activation alone can increase skin permeability and surface evaporation, thus transepidermal water loss (TEWL) is greater from warm skin than from cold one (Cartitlidge, 2000).

1.7.5 Regional variation

Percutaneous absorption in humans is influenced by the area of the body on which the drug is applied. *Stratum corneum* (SC) in some areas like the palms of the hand and soles of the feet may be thicker than other areas in the human body, thus, in general the penetration of solutes is slower through these regions than other parts of the body. For instance, scopolamine transdermal systems are sufficiently enhanced to deliver effective quantities of drug into the body when applied in post auricular area (behind the ear). (Bronaugh, 1999)

1.8 The process of percutaneous absorption

When a drug system is applied topically, percutaneous absorption involves two steps (Hosney , *et al* , 1998):-

1-Releasing the drug from the delivery system

2- Penetration of the released drug through the statum corneum (SC) which is usually the rate limiting step for transdermal delivery (TD).

The process of transdermal penetration includes many routes for transportation (Figure1.4). The solubility and diffusivity are the two determinants for a solute crossing a membrane. The solubility of a solute in two phases sets its partition coefficient and therefore determines the permeability of the solute that is released from a vehicle into the SC. On the other hand the solubility also helps determining whether a solute is likely to be desorbed from the outermost layers into deeper layers. The diffusivity gauges the speed at which a solute crosses a given barrier. (Riviere, 2006).

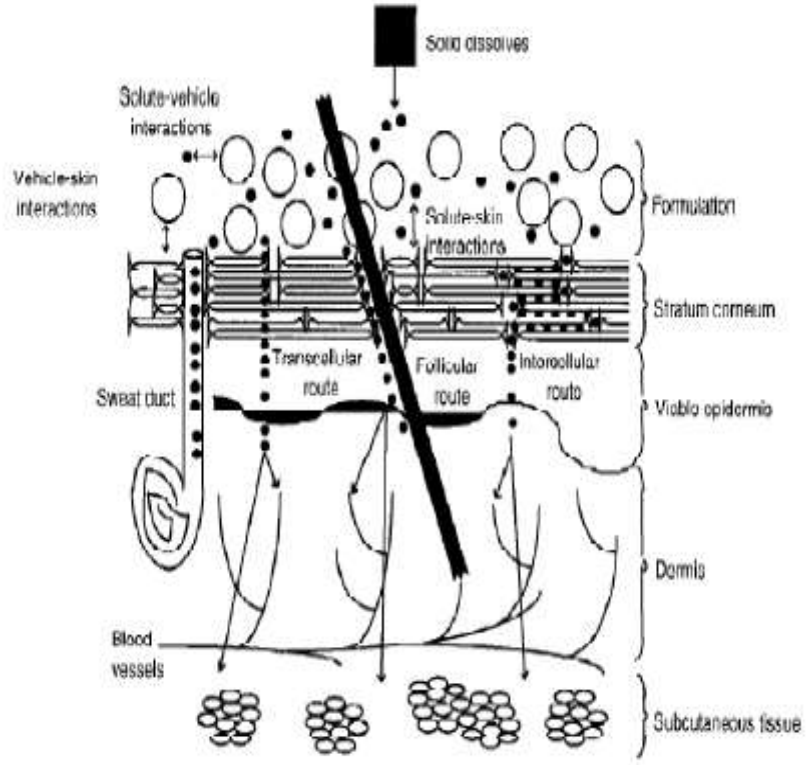


Figure1.4: "Schematic representation of the processes contributing to the permeability of a solute through the skin to the bloodstream or underlying tissues."(Riviere,2006).

1.9 Routes of penetration through human epidermis

Once the drug is applied topically it is released from the vehicle, and is believed to passively diffuse through the skin by three potential pathways or routes: (Suhonen, T,*et al*,(1999),(Williams,Barry,2004)

-Sweat ducts.

-*Stratum corneum* (SC).

-Hair follicle with their associated sebaceous glands. Fig. (1.5) illustrates the three potential pathways or routes.

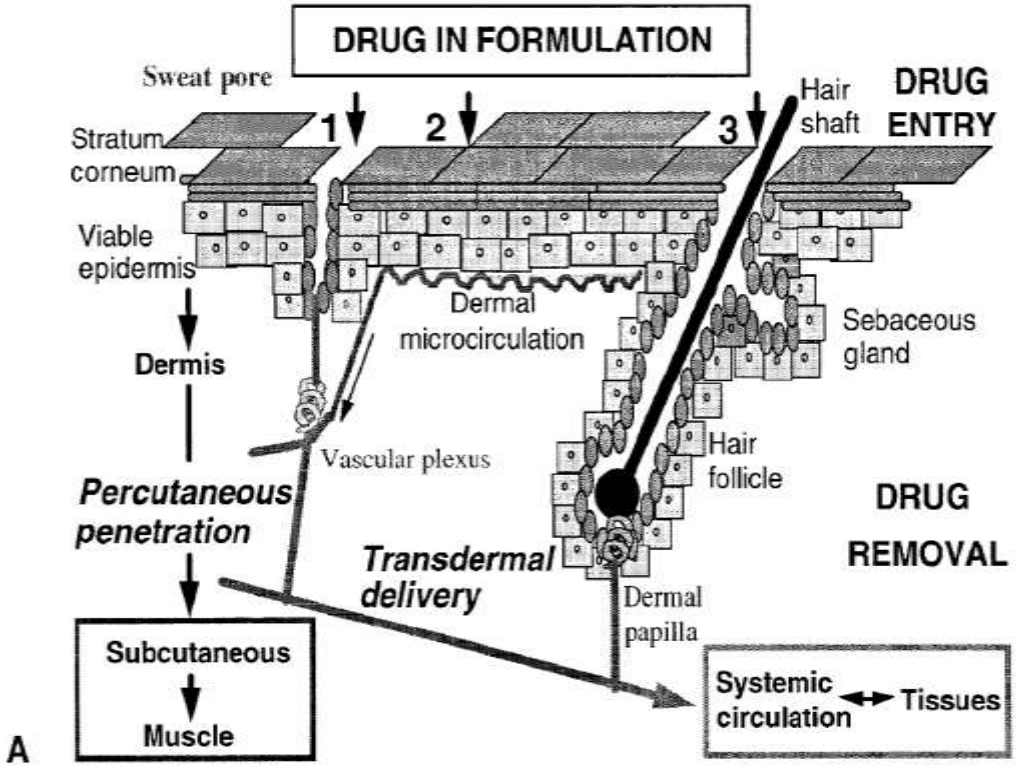


Figure 1.5: routes of penetration through human skin. (Walters,2002).

1.9.1 Hair follicles , & Sweat glands (Skin appendages)

Sweat glands, hair follicles, plus sebaceous glands can facilitate the penetration of a drug across the skin. These appendages , however , occupy only about 0.1% of the overall human skin area , this causes their effect on the total diffusion to be limited , but these trans-appendageal shunt routes can play a significant role in

the permeation of molecules with large sizes that cannot permeate through the SC.(Suhonen, *et al*, 1999). The trans-appendegeal routes have a shorter lag time and a greater diffusivity than transcellular transport. Thus this route may predominate for some period especially after the initial stage of topical application and after that the transcellular transport predominates. (Ho,2004).

1.9.2 *Stratum corneum* route:

The *stratum corneum* is considered a very effective barrier against permeation of drugs into the human skin, and it provides the rate limiting stage of permeation. (Ramachandran,Fleisher,2000).

The S.C is often visualized as brick wall structure with mortar in between, the protein (corneocytes) forming the bricks and the intercellular lipid matrix forming the mortar. There are two potential pathways for drug permeation theough the *stratum corneum* as illustrated in (Fig. 1.6) (Ho,2004) , (Lin,*et al*,1996):

They are the intercellular lipid pathway between corneocytes and the transcellular route.

1.9.2.1 Intercellular Diffusion:

In the intercellular space the lipids of the *stratum corneum* is arranged into a double bilayer between corneocytes.(Wickett,Vsscher,2006).This arrangement of multiple bilayers or lamellar structures may be formed as a result of interaction between water & free fatty acid which is partially saponified at the intermediate pH of the skin (Friberg , *et al*,1987). Altering of the phase structure of the intercellular lipid matrix, lead to altering the barrier properties of lipid bilayer and thus affect the drug permeation through the *stratum corneum*. We can use a group of chemicals called penetration enhancers that can be inserted between the hydrophobic tails of the bilayer causing an increasing in their fluidity, disturbing their packing which will result in easier diffusion of penetrants. (Suhonen, T,*et al*,(1999). Fig (3.7) illustrates the action of PE in the intercellular lipid domain of the *stratum corneum*.

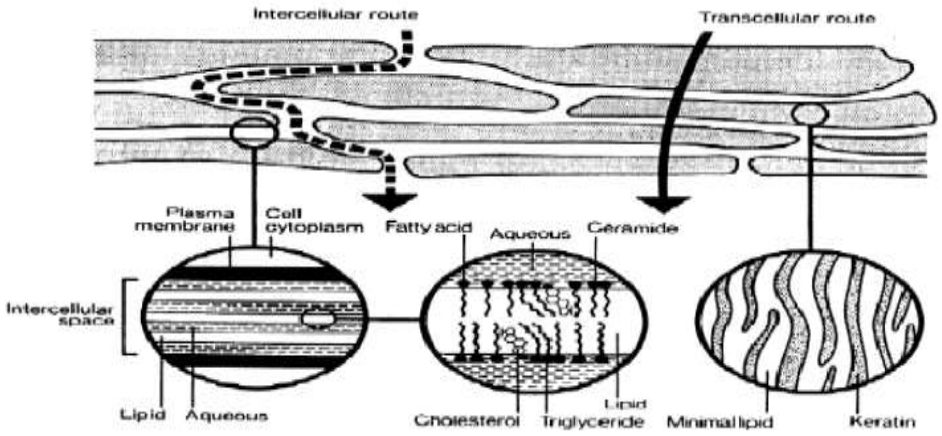


Figure 1.6: "A scheme representing proposed routes for drug permeation into the stratum corneum (i) the transcellular pathway (across the corneocytes) and (ii) the intercellular pathway "via the lipid matrix between the corneocytes)". (Suhonen,T,*et al*, 1999).

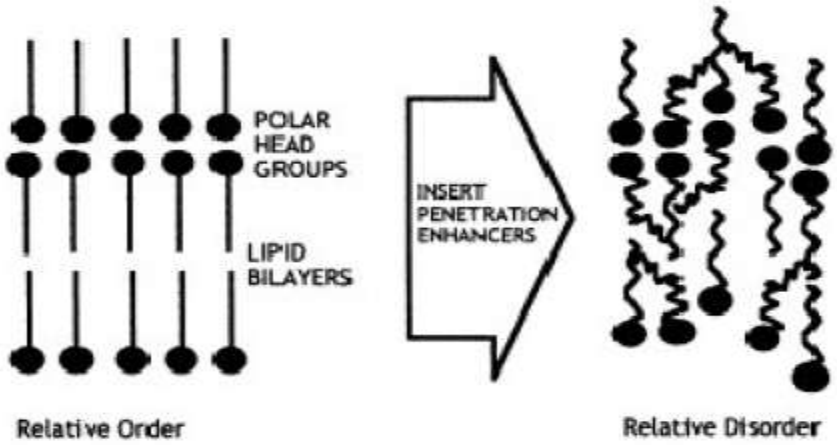


Figure 1.7: A scheme representing how PEs act in the intercellular lipid domain of the *stratum corneum*. The figure clarifies the change from normal order of the intercellular lipids to relative disorder as a result of the action of the enhancers. (Suhonen ,T ,*et al*,1999).

1.9.2.2 Transcellular Route:

The drug permeation must occur through the dense protein structure that forms a significant barrier against permeation. There are two types of keratin in the stratum cormeum one of them is called type I keratin (acidic) and the other is type

II keratin (neutral to basic). Difference between the acidic proteins and the basic one is that acidic protein have more negatively charged amino acid side chains (aspartic or glutamic acid), while basic proteins contain plenty of positively charged amino acid side chains (lysine, arginine , or histidine at low pH). This causes the two parts of proteins to interact with each other forming the coiled-coil structure. This arrangement plays an important role in the keratinocyte structure and help keeping its totality. (Wickett,Vsscher,2006). The permeants diffuse across the corneocytes as well as the intercellular lipid. (Sugarman, 2008). Under normal conditions the intercellular spaces is the predominant route, this was approved experimentally. (Ho,2004).

1.10 Permeation enhancement

Stratum corneum act as the main a barrier for delivery of therapeutic agents transdermally .To reduce these difficulties, researchers have employed several approaches

1.10.1 Chemical penetration enhancers:

Penetration enhancers are skin promoters or accelerants which are substances that simplify and facilitate the solutes absorption across biological membranes. (Williams,Barry,2004).

1.10.2 Prodrugs

A pro-drug is a compound that is metabolized after administration into a pharmacologically active drug. Inactive **pro-drugs** are pharmacologically inactive compounds that are metabolized into an active form in the body. Instead of administering a drug directly, a corresponding **pro-drug** might permeate better through the skin barrier and later may be converted to its active form. (Ho,2004).

1.10.3 Electroporation:

The application of a series of short, high-voltage pulses to the skin causing temporary pores in skin barrier that facilitates the transportation of molecule

across the skin. Electroporation utilize the implementation of high voltage (e.g. 5-75V) for a short time to the *stratum corneum* which causes the formation of new permeation pathways through multi-lamellar lipid membranes.(Ting, *et al*,2004).

1.10.4 Iontophoresis:

In this approach, two electrodes are applied to the skin and connected to a battery. When an ionized drug is in contact with one electrode migration will occur under the influence of the voltage gradient through the skin forcing the drug to enter the systemic circulation. (Benson, 2005).

1.10.5 Ultrasound

Ultrasound applies sound waves to the skin with a frequency of more than 15 to16 kHz which causes waves to travel. This pressure variations cause mixing, forming of cavities and increase in temperature which can increase the permeation of a drug. (Ho.2004)

The most popular method is using a chemical penetration enhancer.

1.11 Ideal Chemical Penetration Enhancers (Williams, Barry, 2004).

Ideal Penetration Enhancers have the following characteristics:

1- They should not be toxic, irritant or allergenic.

2- They must be pharmacologically inert, preferably not interacting with receptors in the skin

3- They must work very fast also the activity and duration of effect should be expected.

4- Penetration Enhancers should allow a drug into the body without losing body fluids and electrolytes which means that they should work unidirectional.

5- The skin should immediately return to its normal barrier property.

6- They must be compatible with all ingredients additives (excipients) and should be acceptable for cosmetic preparations with a suitable skin feel.

- It is not easy to find an ideal P.E that has all these characteristics together.

1.12 An overview of some Penetration Enhancers

1.12.1 Water:

The *stratum corneum* of human contains nearly 16–20% water as a dry weight. About 30 % of the water which is present into the *stratum corneum* can be considered as 'bound'. The rest of it is free and act as solvent inside the membrane for polar permeants. Also human skin have a hygroscopic humectant mix of amino acids and salts known as the natural moisturizing factor (NMF).These components retain the quantity of water in the *stratum corneum* and acts to preserve tissue pliability. Beside that corneocytes are filled with keratin carrying (functional groups) such as -OH and –COOH that also play a role in binding water molecules within the tissue. (Williams, Barry, 2004). It is difficult to understand mechanisms of action by which water enhance transdermal drug delivery; it is proposed that the permeate solubility and partitioning in the *stratum corneum* may be altered by the presence of free water. Swelling of skin occurs as a result of occlusion or soaking and hence disruption of lipid bilayer structures by distention of the polar regions within the bilayers or swelling of the corneocytes. Collection of information obtained from "freeze fracture electron microscopy "of fully hydrated *stratum corneum*, shows that the intercellular lipid bilayers have (water pools or vesicle like structures) within. In general, more hydration for tissues seems to improve transdermal delivery of both hydrophilic and lipophilic permeants (Williams, Barry, 2004). Some environmental effect, like low humidity, sun, wind and surfactants can cause lowering the SC water content, this can harm the function of enzymes needed for desquamation causing cohesion and aggregation defects of corneocytes which can be seen as dryness, roughness, scaling, and flaking. (Verdier-Sévrain ,Bonté, 2007)

1.12.2 Sulphoxides and similar chemicals:

Dimethylsulphoxide (DMSO) is an early discovered a penetration enhancer. Other examples are Dimethylacetamide (DMAC) and dimethylformamide (DMF) which considered powerful aprotic solvents with structures similar to that of DMSO. They denature the intercellular structure or promote lipid fluidity by disruption of

the lipid domain.(Williams,Barry,2004). Figure (1.8) illustrates the chemical structure of Sulphoxides.

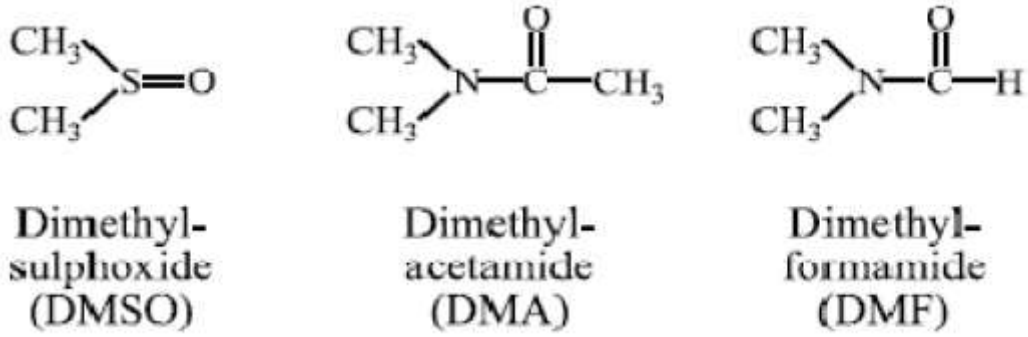


Figure 1.8: (Aprotic solvents that act as potent penetration enhancers.)

1.12.3 Azone:

The first molecule especially designed as a penetration enhancer for skin is Azone(1-dodecylazacycloheptan-2-one or laurocapram). (Figure1.9). It is a very lipophilic compound, It is not irritant, has very low toxicity with only a little pharmacological activity. It interacts within the lipid domains of the stratum corneum. The chemical structure of Azone has a big polar head group and a hydrophobic alkyl chain; it is expected that the penetration enhancer partition inside the bilayer lipids to damage their arrangement. (Williams,Barry,2004).

In general fluidization of the intercellular lipids and alteration of protein conformation is the most common mechanism of action of Azone. (Lambert,*et al*.1989). Azone can be used with many compounds and it has been demonstrated to enhance the penetration of both polar and non-polar drugs through SC. (Hosney , *etal* , 1998)

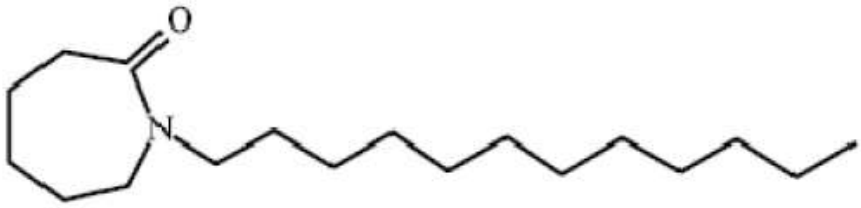


Figure (1.9) Azone, the first molecule to be synthesized so as to act as a skin penetration enhancer (Williams,Barry,2004)..

1.12.4 Fatty alcohol, alcohols, and glycols

Alcohols like ethanol has been proposed to make modification to the lipid bilayers (the polar head group region), also ethanol in high concentration usually extract lipids from S.C. Fatty alcohols (or alkanols) for example 1-octanol , 1-propranolol and 1-butanol may have penetration enhancing effect that is increased by increasing the number of carbon atoms. PG shows synergistic action when it is used as vehicle for penetration enhancers such as oleic acid and ethanol, PG penetrate widely through the *stratum corneum* and its mechanism of action may be due to the solvation of keratin within SC and hence disruption of SC structure. (Walker,Smith, 1996), (Williams, Barry,2004).

1.12.5 Surfactants:

Sodium lauryl Sulphate (SLS) is an anionic surfactant, whereas cetyltrimethyl ammonium bromide is cationic surfactants, nonionic surfactants include nonoxynol, "zwitterionic surfactants" include dodecyl betaine. Surfactants which are anionic and cationic may be irritant to the skin of humans. The mechanism of action for these compounds is adsorption at interfaces and interaction with biological membranes. Anionic surfactants may act by changing the function of the barrier of SC layer after producing large alterations in the barrier properties (Walker,Smith,1996).

1.12.6 Fatty acids:

Saturated Fatty acids composed of 10 to 12 carbons give strong enhancement and unsaturated fatty acids with 18 carbons appears to have also good permeation enhancement effects, the *cis* configuration is believed to be more active than the *trans* configuration. These effects come as a result of disruption of intercellular lipid packing. Oleic acid is the most popular one. (Williams, Barry, 2004).

1.12.7 Urea:

Urea is usually used in the treatment of psoriasis, it is a hydrating agent. It combined with Salicylic acid for the treatment of keratolysis. It increases

permeation of drugs by facilitating hydration and forming hydrophilic channels within SC (Walker,Smith,1996).

1.12.8 Essential oils, terpenes and terpenoids:

Terpenes are naturally occurring compounds containing only carbon, hydrogen and oxygen atoms, they are found in essential oils. They are considered as safe and clinically acceptable accelerants for both hydrophilic and hydrophobic drugs. Synergistic effect has been shown when they are in combination with PG. Nerolidol, d-limonene,1-8-Cineole,and carvone are examples of these compounds. (Moghimi, *et al*, 1996).

1.13 Solvents at high concentrations

Elevated concentration of solvents may cause plenty of severe effects. Those may harm desmosomes, causing splitting of the- intercellular lipid- and fissuring of the SC.

Solvents could get in the corneocyte, extremely deactivating keratin and even vacuoles may be formed. (Williams, Barry, 2004). Figure (1.10) illustrate Dramatic action of high concentration of enhancers.

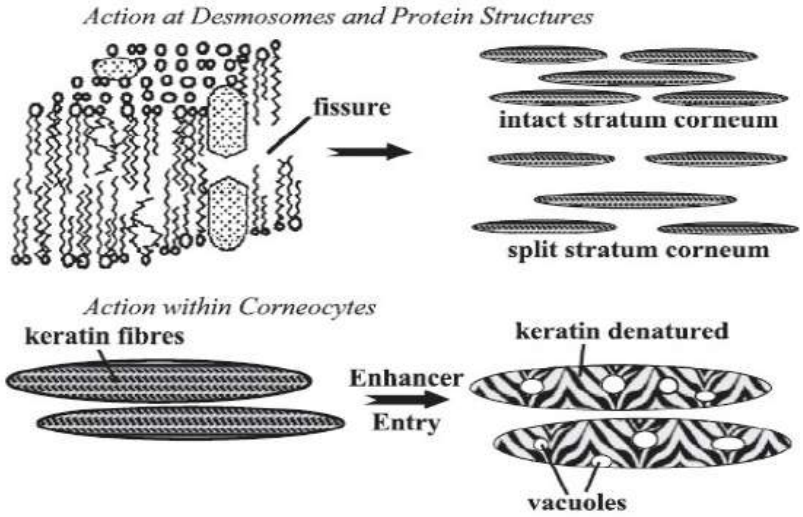


Figure 1.10:" Dramatic action of high concentration of penetration enhancers (particularly solvents) on the integrity of *stratum corneum* adhesion." (Williams, Barry, 2004)

1.14 Mechanism of action of P.Es

The methods by which the enhancers act are complex in general, and include the following (Williams, Barry, 2004):

- Disruption of the barrier features of the *stratum corneum* .
- Interaction with intarcellular lipids of the SC.
- Increasing the drug partitioning into the stratum corneum.

Figure (1.11): "illustrate how the mechanism penetration enhancers within the intercellular lipid domain."

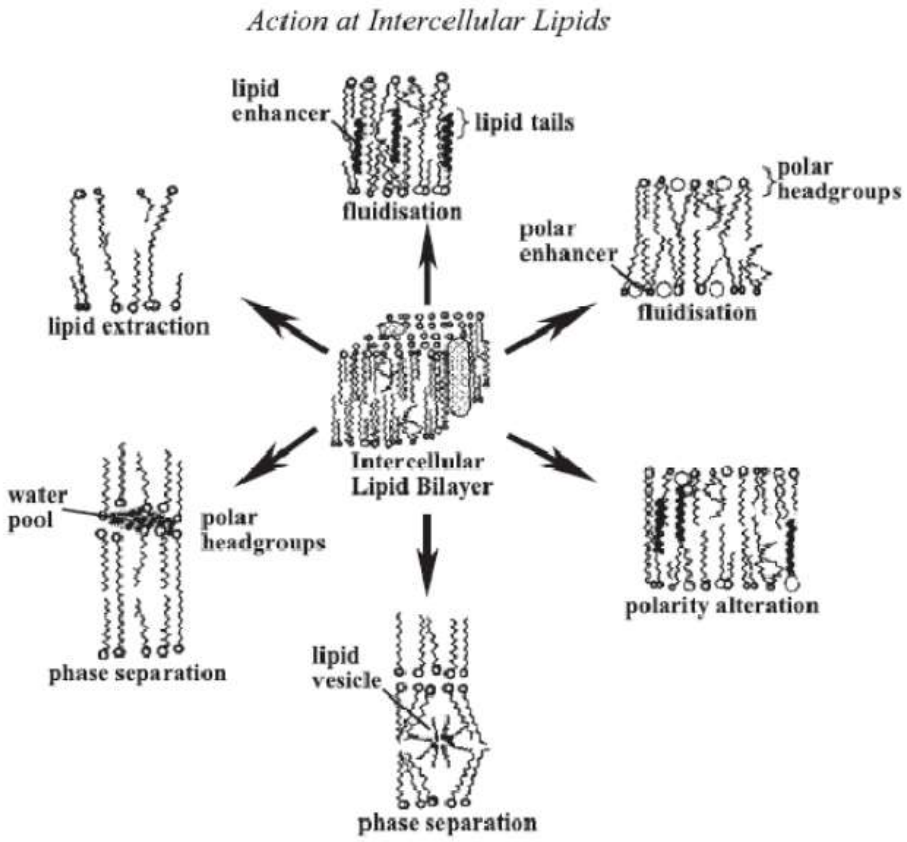


Figure 1.11: "Actions of penetration enhancers in the intercellular lipid domain." (Williams,Barry, 2004)

1.15 Topical gel preparation

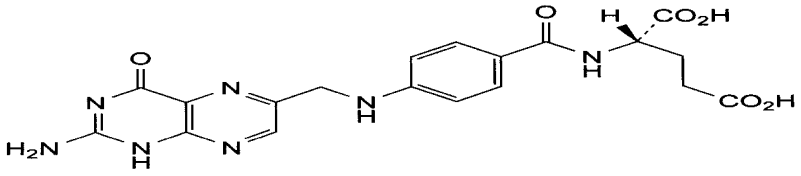
Gels are semisolid, transparent non greasy aqueous preparations, that is formed by trapping liquid phase at the interlocking of the three dimensional polymeric matrix of a synthetic polymer or a natural gum.

It takes only 0.5-2% of the most commonly used gelling agents to setup the systems (Banker, Rhodes, 2002).

The advantage of gel system over other preparations is due to their very low solids content which **(1)** will decrease the binding ability of the drug to the matrix of gel and **(2)** it will prevent prolongation of the drug diffusion related to obstruction by the gel matrix, moreover a gel may seem to be quite viscous macroscopically, the resistance it gives to a diffusing drug molecule may be similar to that provided by a less viscous liquid phase. (Ramanathana, Blockb, 2001). An example of gel systems that can be used for transdermal, ocular buccal, rectal, and nasal applications is Carbopol. The drug release rates of the HydroxyPropyl Methyl Cellulose (HPMC) gels are so sensitive to the rheological behavior of the topical gel formulations. (Islam, *et al*.2004)

1.16 Folic Acid

Folic Acidchemically, is -(2S)-2-[[4-[[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl)methyl] amino]benzoyl]amino] pentanedioic acid- , and has the following Chemical Structure:



$C_{19}H_{19}N_7O_6$ Mwt =*441.4*

Fig. 1.12: Structure of Folic Acid (BP 2017).

"It is odorless, tasteless and yellowish to orange, crystalline powder; practically insoluble in water and in most organic solvents. It can be dissolved in diluted acids and also in alkaline solutions. [BP2013]."

Table 1.1: Chemical and physical properties of folic acid [BP2017]:

Chemical name	{(2*S*)-2-[[4-[[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl) methyl] amino] benzoyl] amino]pentanedioic acid.}
Appearance	{Orange or yellowish , crystalline powder.}
Solubility	{It is insoluble in water and in most organic solvents. But folic acid dissolves in dilute acids and in alkaline solutions.}
Specific optical rotation	+ 18 to + 22 (anhydrous substance)
Sulfated ash	Maximum 0.2 %

Its aqueous solutions are heat sensitive and decompose rapidly in the presence of light. Folic Acid has two pKa, i.e. pKa = 3.5 and 4.3 (carboxylic acid moieties). Folic Acid is available in the market in tablet dosage form as single Folic acid tablets or in combination with iron compounds and other B vitamins. (Thomson PDR 2009).

Therapeutic indications

Folic acid is one of the B group vitamins and plays a role in the production and maturation of red blood. It is generally indicated in the following cases (Combs GF Jr. 2012) :

1. For the treatment of anemias like megaloblastic anaemia (folate-deficient), celiac disease also may cause its deficiency.(Aslinia, F, Mazza JJ, Yale SH. 2006)
2. Indicated for anemia caused by administration of phenytoin, phenobarbital and primidone (Kasper, DL, Fauci AS, Longo DL, et al. Eds. 2005).
3. Indicated for the prophylaxis against deficiency of folate progressed in renal dialysis or chronic hemolytic cases (Berkow R., ed. 2003).
4. Indicated for preventing neural tube defects, in women whom are planning for pregnancy (Bennett JC, Plum F.,1996):.)

Pharmacokinetic

(Chanarini, Mollindl, Anderson BB, 1958) (Baker H, et.al, 1965)

Absorption

Folic acid is absorbed quickly from the GIT, mostly from the" proximal part "of the small intestine. Dietary folate is declared to have nearly half the bioavailability of the crystalline folic acid. The naturally occurring "folate polyglutamates" are broadly reduced by the enzyme, dihydrofolate reductase present in the intestine in order to create "5-methyltetrahydrofolate (5MTHF)". Folic acid which given orally enters the portal circulation mainly unchanged, because it is a poor material for reduction by the enzyme (dihydrofolate reductases.)

Distribution

During circulation," 5MTHF" from naturally occurring folate is widely bounded to plasma protein. Liver is the storage site of folate, also folate concentration is high in CSF and is distributed into breast milk.

Metabolism

Folic acid is converted in the liver and plasma to "5MTHF" which is metabolically active. Folate has an enterohepatic circulation.

Elimination

The elimination of" Folate" metabolites occur via urine, excess folate in the body is excreted without being changed.

1.17 Semisolid Drug Products Quality and Performance Tests

Drug product tests are divided into two categories According to (USP 40):

1-Tests that assess general quality features,

2- Tests that assess product performance, e.g releasing of the drug substance in vitro from the drug product.

Quality tests, such as description, identification, assay, impurities, pH, microbial limits, antimicrobial preservative content, crystal growth and viscosity assess the integrity of the dosage form while Performance tests, such as drug release, assess attributes that relate to in vivo drug performance.

1.17 In vitro diffusion cells

Vertical Franz Diffusion cells are a credible and reproducible means in order to measure the release of drug from semisolid dosage forms. Figure (1.13) illustrates the components of Vertical FDC (USP 40, 2017).

Franz diffusion cell is composed of (Kamil, 2006):

1- Donor chamber

2- Receiver chamber

3- One sampling port

4- Water jacket

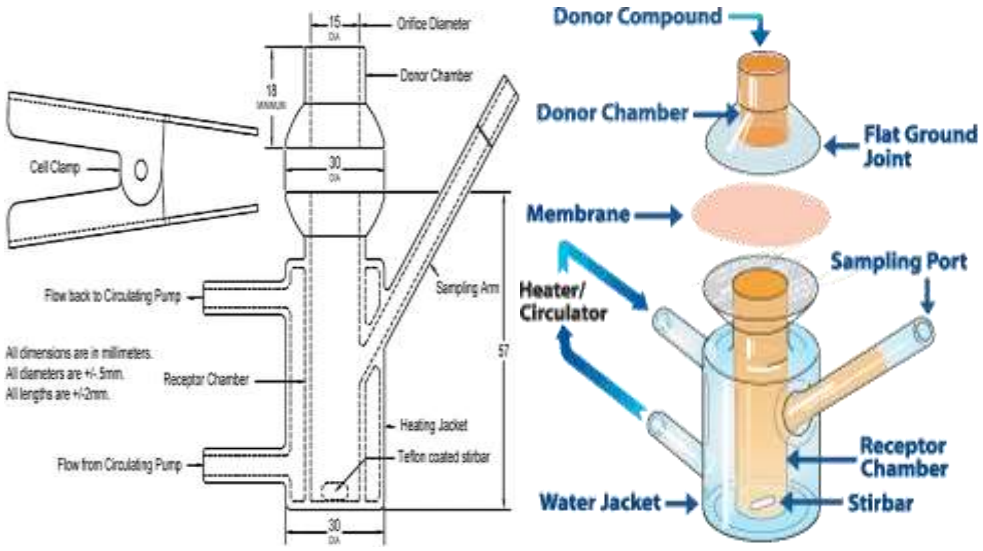


Fig. 1`.13 Vertical Franz Diffusion Cells (USP 40):

The donor and receiver chambers are separated by membrane. A thick layer of the semisolid product under evaluation should be placed in contact with a medium in a reservoir, and the later works as a receptor when the drug substance diffuses through the formulation, across the membrane, and into the reservoir. Diffusion occurs across an inert, highly permeable support membrane.

For quality control function the membrane is planned to retain the product and the receptor medium separated and distinct, Synthetic membranes employed in FDC experiments include Regenerated Cellulose (RC), Cellulose esters (CE), Cellulose nitrate (CN), Polyacrylonitrile (PAN), Polyamide (nylon) (PA), Polyether sulfone (PES), Polysulfone (PS), Polycarbonate (PC), and Polypropylene (PP) (Shiow-Fern, 2010).

For drug permeation studies human skin is used. Difficulties in obtaining and using human skin have tempted many workers to employ:

a. Hydrophobic synthetic membranes, such as Polydimethylsiloxane (PDMS) as rate limiting and imitating the *stratum corneum* (Shiow-Fern, 2012),
b. Animal skins as model membranes, such as a skin of snake, smooth mouse skin (hairless) and pig skin (J. Invest. Dermatol. 94. 235-240, 1990).
c. Natural membranes like inner layer of egg of Gallus Domesticus (Hen), tomato, peach and onion (Roshan Nawale, 2013).

1.18 Principles of diffusion through membrane

Diffusion is a mass transfer of individual molecules of a substance due to Concentration gradient by random motion .This diffusion through the SC is a passive one. (Martin, *et al*.1983)

According to Fick's First Law, the amount of material, M, that flow over a unit cross section, S, of a barrier in unit time, t, is known as the flux, J.

$$J = \frac{dM}{S \cdot dt} \quad \text{.......} \quad (1)$$

The flux, J, is proportionate to the concentration gradients, dc / dx :

$$J = -D\frac{dC}{dx} \quad \text{.......} \quad (2)$$

Where, D is representing the diffusion coefficient of penetrant (also called the diffusant) in cm^2/sec, c represent the penetrant concentration in g/ cm3,and x is the distance coordinate in cm.

The negative sign of this equation indicate that diffusion or flow in the direction of lowering concentration of diffusant, the diffusion coefficient, D ,or diffusivity as it's often called, does not stay constant. D depends on Pressure, temperature, concentration, solvent properties, and the diffusant chemical nature, so, D is more correctly represented to as a diffusion coefficient instead of a diffusion constant.

In the initial stage of diffusion, the molecules of the drug may penetrate the skin appendages (hair follicles, sweet gland) then diffusion through S.C becomes dominant when a steady state has been accomplished (Moammal, Q. 2009).

"Fick's Second Law" of diffusion examines the diffusant concentration change with time at a specific point in the system. An equation for mass transportation which discusses the alteration of concentration with time at a specific site, instead of the mass diffusing across unit area of barrier in unit time can be seen at equation 3.

$$\frac{\partial C}{\partial t} = -\frac{\partial J}{\partial x} \quad \text{..... (3)}$$

Change in concentration is caused by variance in and output and input .That is the concentration of penetrant in the volume element alters with time as the flux or amount diffusing differ with distance x.

Differentiating the 1st law expression from equation (2), with respect to x ,we get:

$$-\frac{\partial J}{\partial x} = D\frac{\partial^2 c}{\partial x^2} \quad \text{..... (4)}$$

Substituting from equation (3) into equation (4) gives in Fick's 2nd law

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \quad \text{..... (5)}$$

A steady state is an important condition in diffusion process, Ficks 1st law, equation (2), gives the flux/area in steady state conditions of the flow. The 2nd law mention the change in concentration of diffusant with time at any distance, x.

Ficks may be written as follows (Patrick J. Sinko,2011):

$$J = \frac{dM}{S \cdot dt} = D \left(\frac{C_1 - C_2}{h}\right) \quad \text{..... (6)}$$

Where, S is the membrane area and, h is the membrane thickness. C1 and C2 represent the concentrations within the membrane boundaries; they are not recognized but they can be substituted by the partition coefficient K multiplied by the concentration of permeant in the donor phase C_d, or in the receiver C_r as follows:

$$K = \frac{C_1}{C_d} = \frac{C_2}{C_r} \quad \text{..... (7)}$$

So, from equation (6),

$$\frac{dM}{dt} = \frac{DSK\,(C_d - C_r)}{h} \quad \text{..... (8)}$$

If sink conditions hold in the receptor phase, that is C_r =0, that will result in equation 9

$$\frac{dM}{dt} = \frac{DSK\,C_d}{h} = PSC_d \quad \text{..... (9)}$$

In which P is the permeability coefficient that is given by equation 10:

$$P = \frac{DK}{h} \text{ cm/hr} \quad \text{..... (10)}$$

We measure the cumulative amount of diffusant, m, that passes per unit area through the membrane as a function of time and we obtain the plot shown in Figure (1.14)

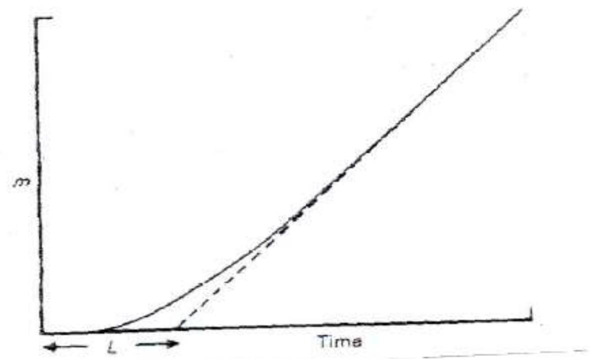


Figure (1.14): The time course of absorption for the zero flux case.(Patrick J. Sinko,2011)

After prolonged times the plot has a straight line and a steady state flow is obtained. Intercept with x axis gives the lag time, $T_{L,}$ which can be expressed by the following relation

$T_{L=}$ $h^2/6D$……………………………………..…(11)

Part Two
Objectives and Significance of the Study

2. Objectives and Significance of the study

2.1 Objectives of the study

1- Preparation of topical Folic acid gel.

2- Investigation the effects of different penetration enhancers on the drug permeation rate through a natural membrane using Franz –type diffusion cell.

To complete the project, the following stages should be carried out:

1- Analysis of API (Folic Acid) according to BP (for raw material) and USP test methods

2- Solubilization of Folic Acid in different solvents, solvent mixtures and buffers to select the appropriate ones in the preparation of gel and/ or in the diffusion studies

3- Compatibility study: The purpose of this stage is to find compatible excipients with the active ingredient (Folic Acid) for the preparation of 0.1% gel

4- Preparation of Gel formula that contains 0.1% of Folic acid and different penetration enhancers.

5- Study the permeability behavior of Folic acid gel with the penetration enhancers under test using Franz Diffusion Cell (FDC) through natural membrane.

6- Select the most appropriate penetration Enhancer (PE) for further study

7- Study the permeability behavior of Folic acid gel with the selected penetration enhancer using Franz Diffusion Cell (FDC) through Pig skin

8- Stability study of the Folic Acid gel.

9- Analysis of data to determine the amount of drug that penetrated the membrane during previous experiments.

2.2 Significance of the study

DNA damage in human skin caused by environmental factors such as ultraviolet radiation is a major contributor to skin aging

Photo-aged skin proceeds in one direction and characterized by:

- coarse wrinkles,
- loss of elasticity,
- pigmented spots,
- dryness,
- verrucous papules;

Folic acid plays an important role in life process of mitotically active tissues and its deficiency increases background level of DNA damage. Folic acid seems to have skin regeneration properties and it can modulate DNA repair in UV-damaged skin.

In vivo tests showed that a cream containing folic acid improved the skin elasticity and moisturizing, decreased trans-epidermal water loss and skin roughness without any significant change of sebum secretion (Lewis D. A.,2011).

The B vitamins help slowing down premature skin ageing. The well-known vitamin B5 (panthenol), Vitamin B3 (niacinamide) and Vitamin B6 (pyridoxine HCl) have been used for years in personal care products for their benefits for skin and hair.

Only vitamin B9 (folic acid) remained the unstudied for cosmeceutical applications. (Maru G. B., Gandhi K., Ramchandani A., Kumar G.)

Part Three

METHODOLOGY

3.1. Materials and Reagents

All materials used in the study were of pharmaceutical grade (Table 3-1), and all reagents were of analytical grade (Table 3.1). These materials and reagents were purchased from reliable sources and donated by Jerusalem Pharmaceuticals Co, Ltd.-Ramallah Al Bireh-Palestine.

Table (3.1) the materials and reagents used in the study of Folic acid gel

Purpose	No.	Item	Description	Function
Formulation	1	Folic Acid	BP	API
	2	Benzyl Alcohol	BP/USP	Preservative
	3	CARBABOL 940	BP/USP	Gelling Polymer
	4	Disodium Edetate	BP/USP	Synergistic Antioxidant
	5	Polyoxyl-15-Cetostearyl Ether	BP/USP	Solubilizer/ Penetration Enhancer
	6	PROPYLENE GLYCOL	BP/USP	Humectant
	7	PURIFIED WATER	BP/USP	Vehicle
	8	SODIUM LAURYL SULPHATE (SLS)	BP/USP	Solubilizer/ Penetration Enhancer
	9	Tween 20	USP/NF	Solubilizer/ Penetration Enhancer
	10	Triethanolamine (TEA)	USP/NF	Alkalizing agent
	11	Isopropyl Alcohol (IPA)	USP/NF	Penetration Enhancer
	12	Isopropyl Myristate (IPM)	USP/NF	Penetration Enhancer
Packaging Materials	1	Aluminum Tubes	Manufacturer – Lageen	Primary Packaging Material
Analytical	1	Dibasic Sodium Phosphate	Merck	Reagent
	2	DIMETHYL SULPHOXIDE	AR	
	3	ETHANOL	AR	Reagnt
	4	ETHYL ACETATE		
	5	METHANOL		Reagent
	6	Monobasic Potassium Phosphate	AR	Buffering agent
	7	WATER-PROOF PARAFILM	AR	Sealing
	8	n-OCTANOL	AR	Lipid layer simulator
	9	Phosphoric Acid 85%		Acidifying agent
	10	Sodium Acetate	AR	Buffering agent
	11	Glacial Acetic Acid	AR	Buffering agent
	12	Isopropyl Myristate (IPM)	BP/USP	Lipid layer simulator

3.2 **Equipment and Tools:**

Syringes, vials, pipettes, glassware, stands and tubes were supplied by Jerusalem pharmaceuticals. Tools and equipment used in the study are listed in Table (3.2).

Table (3.2) Equipment and tools used in the study of Folic acid gel.

Equipment	**Source/Model**
HPLC	Merck- Hitachi.
U.V. Spectrophotometer	Hitachi U2900
7 Station Diffusion Cell Apparatus	ElectroLab, Model-EDC-07
pH meter	Metrohm
Percision Balance	Percisia
Magnetic Stirrer	Fried Electronic
Incubator 25C°	Firlabo
Incubator 30 C°	PGC Walk-in Climatic Cabinet
Incubator 40C°	Firlabo
Water Pump	Atman At-101
Synthetic membrane filter PA 0.22 and 0.45µm	Millipore
Water bath	Tuttnauer Co. LTD
Vaccum filter	Sartorious
Sonicator	Elmasonic
Refrigerator	L.G.
Vaccum pump	Millipore
Brookfield Digital Viscometer DV-II + Pro	Brookfield engineering laboratories, Inc.
MALVERN Particle size Analyzer	Model: Mastersizer /Scirocco 2000 Manufacturer: Malvern

3**.3 Analysis of Folic Acid**:

Folic acid used in the study was analyzed according to BP test methods. Tests required and their acceptance criteria are indicated in Table 3.3.

Table (3.3) Specifications of Folic Acid according to BP

Sr#	Test	Acceptance Criteria	Reference
1	Content of Folic Acid	96.0-102.0% (on dried basis)	BP-2017
2	Characteristics	Yellowish orange micronized powder	
3	Solubility	Practically insoluble in water and in most organic solvents. It dissolves in dilute acids and in alkaline solutions.	BP-2017
4	Identification	A. Specific optical rotation: + 18 to + 22 (anhydrous substance). B. The principal peak in the chromatogram obtained with the test solution in assay is similar in retention time to the principal peak in the chromatogram obtained with reference solution	BP-2017
5	Water content	NMT 8.5%	BP-2017
6	Sulfated ash	NMT 0.2%	BP-2017
7	Related Substances	NMT 0.5% of impurity A NMT 0.6% of impurity D NMT 0.5% of any other impurity NMT 1.0% of total impurities	BP-2017
8	Particle Size	D90 should be less than 20 µm	In-house

3.4 Solubility determination

To determine the solubility of Folic Acid in water and in different pH conditions, Folic Acid was added in excess amount to separate glass vials type I containing:

1- Water
2- Acetate Buffer with pH=4.5
3- Potassium dihydrogen phosphate Buffer with pH=6.8
4- Potassium dihydrogen phosphate Buffer with pH=7.4
5- 0.1% Triethanolamine

Each vial was shaken for about 4 hours at ambient 25 C º, sonicated for 10 minutes and filtered from excess Folic Acid through 0.45µm nylon filter membrane. The clear solutions containing soluble Folic Acid were then analyzed spectrophotometricaly to determine the amount of soluble Folic Acid (United States Pharmacopeia Convention, 2017) as per Analysis section 3.13. The solubility results were expressed in g/100ml.

3.5 Compatibility study

In order to investigate the compatibility of Folic Acid with the expected excipients and penetration enhancers under study, stock solutions were prepared: Folic Acid (1.0 g) was dissolved in a mixture of triethanolamine (1g) and water (50ml) in a 100-ml volumetric flask and the volume was completed to 100 ml with water.

The Stock Solution was used as a diluent for the preparation of different solutions containing about 0.01% Folic acid and each separate excipient. Table 3.4 illustrates the prepared solutions.

Table (3.4) List of excipients and their Concentrations used in the compatibility study with Folic acid

Sr. #	Excipient	Concentration
1	Benzyl Alcohol	2%
2	Carbopol 940	1%
3	IPM	5%
4	TEA	0.1%
5	Tween 20	1%
6	Propylene Glycol	10%
7	PSE-15	2%
8	SLS	0.3%
9	IPA	30%

Each solution was filled in separate glass vials type I, labeled and closed tightly. The vials were incubated at 40 C º for three days. Samples were pooled and analyzed at initial time and at the end of 3 days. The content of Folic Acid was determined by HPLC method which is described in section (3.13.).

From the assay results of API (Folic Acid), the compatible excipients were determined and the best compatible excipients and penetration enhancers were chosen for further study.

3.6 Preparation of Folic Acid Gel

3.6.1 Composition of formulation trials

Table (3.5) illustrates the general formula for **Folic Acid** Gel where the function of each component is indicated.

Table (3.5) Components of General Folic Acid Gel

NO.	Component	%	Function
1	Folic Acid	0.11*	Active Material
2	Carbopol 940	0.8	Gelling agent
3	Propylene Glycol	5.5	Humectant
4	Disodium Edetate	0.01	Synergistic Antioxidant
5	Benzyl Alcohol	0 or 1	Preservative & Penetration enhancer
6	Triethanolamine	0.8	Alkalizing Agent
7	PE	X	Penetration Enhancer
8	Purified Water	Qs. to 100	Vehicle/Continuous Phase

***: equivalent to 0.1%**

The type and percentage of penetration enhancer used in each formula is illustrated in Table (3.6).

3.6.2 Method of preparation

a. Place Propylene Glycol in a suitable beaker

b. Disperse Carbopol 940 in Propylene Glycol

c. Heat 90% of Water to 85 C

d. Add the content of step [c] to the beaker in step [a] and mix until complete dissolution [Solution A]

e. In another suitable beaker, dissolve Folic Acid in a mixture of residue of water and Triethanolamine. Add Benzyl alcohol, Disodium Edetate and Penetration Enhancer [Solution B]

f. Add [Solution B] to [Solution A]. Mix until a clear gel is obtained. Cool to room temperature.

The pH value of the vehicle is a factor to be considered in the evaluation of drug penetration from gels through membranes or skin (Kushla GP, Zatz JL. 1991). For this reason gel formulations were adjusted to pH 6.4 ± 0.1.

Table (3.6): Formulae of Folic Acid Gel with different penetration enhancers

	Formula	F0	F1	F2	F3	F4	F5	F6	F7	F8
NO.	PE		%	%	%	%	%	%	%	%
1	Benzyl Alcohol	0	1	1	1	1	1	1	1	1
2	IPA	0	0	30	20	0	0	0	0	0
3	IPM	0	0	0	0	2	5	0	0	0
4	Tween 20	0	0	0	0	0	0	0	1	1
5	PSE-15	0	0	0	0	0	0	2	0	0
6	SLS	0	0	0	0	0	0	0	0.3	0

3.7 Permeability behavior of Folic acid gel with the penetration enhancers under test using Franz Diffusion Cell (FDC) through natural membrane

3.7.1 Description of diffusion apparatus:

Electrolab 7 station diffusion cell apparatus was used in the study. Diffusion cells are made of two borosilicate glass components. The upper part is called the cell cap, cell top, donor chamber, or donor compartment. The lower portion is called the receptor chamber. The innermost portion of the cell is jacketed. The apparatus is equipped with temperature controller in the range of 30.0 ºC to 40.0 ºC with controller accuracy of ±0.1 ºC, and with circulating pump with flow rate of 15 liters/minute. The Receptor volume of each cell is 7.5 ml, with 15.0mm orifice /mouth diameter.

3.7.2 The Receiver Phase.

A phosphate buffer with pH 7.4 was used in the receiver compartment to dissolve the Folic Acid that penetrates through the membrane.

The pH of the receiver compartment is the same as the pH of Human blood, pH 7.4. The phosphate buffer was prepared by dissolving 6.8g of KH_2PO_4 in 1000 ml of bi-distilled water. The pH was then adjusted by a solution of 5M NaOH to the desired pH. The receptor phase was then degassed by heating the buffer to 45C^o and vacuum filtration through a PVDF membrane (HVLP filter membrane) with pore size of 0.45µm.

Degassing is very important, in order to keep the receiver phase bubble free when stirring to maintain sink conditions. If not degassed, bubbles will appear due to stirring and will stick under the separating membrane in the FDC. This will lead to a decrease in the permeable area of the separating membrane resulting in decreased permeability and faulty results.

3.7.3 Preparation of chicken Eggshell membrane

The whole chicken egg was added to sufficient quantity of hydrochloric acid solution (about 5M). Wait until the bubbling stops and the foam disappears. The left over substance is eggshell. Eggshell Membrane was prepared by making an aperture at one end of egg, and then yolk was completely removed, the remaining membrane was washed and soaked in n- Octanol or IPM for 2 hours before use.

3.7.4 Diffusion Procedure

An in and out rubber tubes are attached to the inlet and outlet of the water jacket of the FDCs and the receiver compartment of the FDC is fixed place.

A water pump is activated to initiate circulation of warm water through the water jacket of the FDC and back to the interior water bath to maintain a stable temperature of 32±1 ºC.

The receiver compartment is filled to top with the phosphate buffer of pH=7.4 and a magnetic bar is introduced in the compartment. The membrane is mounted on the receiver compartment of the MDC (Habes, 2005), making sure that no bubbles appear. A rubber ring is mounted over the membrane to prevent leakage and the donor compartment is mounted over the rubber ring.

The receiver and donor compartments are now separated by the membrane and a clamp is used to maintain them in their position while the rubber ring prevents leakage due to pressure made by the clamp. When the temperature of receiver phase reaches the assigned temperature range 32 ±1ºC, sufficient quantity of gel (about 5 g) to be tested is introduced into the donor compartment. The receiver compartment, the sampling ports and the receiver-donor junction were covered with Parafilm to assure that no part of the donor or receiver phase is lost.

The stirrer is activated (zero time for the experiment). 1-ml samples are collected from sampling port using a long-needle syringe every hour for seven hours.

After every sample is taken from the cell an equal amount of phosphate buffer pH 7.4 (1ml) is introduced to the receiver compartment of the FDC to ensure that its volume is not affected.

Samples taken were analyzed by HPLC according to test methods (see section 3.13) and every experiment was done in triplicates.

The cumulative amount of the penetrant is calculated according to the following equation:

Cumulative amount of penetrant at time (t) = Ct × V + $\sum_{t=0}^{t-1} Ct$ ….12

Ct: is the measured concentration of the penetrant at time t in the receptor compartment in mg/ml.

V: is the volume of the solution in the receiver compartment.

3.8. Calculation of diffusion parameters.

At every sampling time a sample is withdrawn and the amount of Folic Acid is determined by HPLC analysis, (3.13). A cumulative amount of Folic Acid through time is drawn, and the diffusion parameters are calculated.

The curve is extrapolated using Excel 2007 to find the steady state line. The x intercept of the line will be the lag time. According to equation (8) in section (1.18)

$$\frac{dM}{dt} = \frac{DSK\,C_d}{h} = PSC_d \quad \text{..... (8)}$$

The slope = PSC_d

The thickness of the membrane (h) equals 0.1cm, area of membrane (S) equals 1.77cm^2 and the volume of the receiver compartment is 7.5 ml.

Where S is the area, P is the permeability coefficient; C_d is the concentration in the donor compartment.

The permeability coefficient can be calculated as the slope. The area of membrane and concentration in donor compartment are known.

According to equation (11) in section (1.18):

$$T_L = \frac{h^2}{6D} \quad \text{……..…… (11)}$$

Where h is thickness of membrane that was measured during the experiment, T_L was calculated from the plot so the D the diffusion coefficient is calculated.

According to equation (9)

The permeability coefficient

$$P = \frac{DK}{h} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (10)$$

Where h is thickness of membrane that was measured during the experiment, P is the permeability coefficient that was calculated previously and thus the partition coefficient K is calculated.

A summary of the diffusion parameters and their method of calculation are seen in table (3.7)

Table 3.7: Summary of diffusion parameters and their method of calculation

Slope	Lag Time(TL)	Diffusion Coefficient	Permeability Coefficient	Partition coefficient	Enhancement ratio
Calculated from the plot	Intercept with x axes	$\frac{h^2}{6TL}$	Slope/C_d	$\frac{P.h}{D}$	Permeability with enhancer/ permeability without enhancer

3.9 Selecting the best penetration enhancer

To study the effect of penetration enhancers on the permeability of Folic acid through a natural membrane, several penetration enhancers were mixed with gel of the API and investigated for permeability using MFDC and chicken egg membrane.

The diffusion parameters are calculated and the permeability coefficient of Folic acid gel with the penetration enhancer is compared to the permeability coefficient of Folic acid gel without penetration enhancer to calculate the enhancement ratio. The best penetration enhancer is selected according to the best permeability coefficient value.

3.10 Formulation of Folic acid gel with the best penetration enhancer

Table (3.8): The composition of selected Folic acid gel is described as Formula F1:

NO.	Component	%	Function
1	Folic Acid	0.11*	Active Material
2	Carbopol 940	0.8	Gelling agent
3	Propylene Glycol	5.5	Humectant
4	Benzyl Alcohol	1	Preservative & PE
	Disodium Edetate	0.01	Synergistic Antioxidant
5	Triethanolamine	0.8	Alkalizing Agent
6	Purified Water	Qs. to 100	Vehicle/Continuous Phase

3.11 The permeability behavior of Folic acid gel with the selected penetration enhancer using Franz Diffusion Cell (FDC) through Pig skin

3.11.1 Preparation of Pig Skin

A fresh pig skin sample was obtained from Beit Jala /Palestine, the pig is 10-weeks' old female pig and weighing 25 kg. The whole skin membranes were removed from the underlying cartilage. Hairs were cut and the whole membranes were used immediately (fresh skin) or frozen stored at -18C until further use (frozen skin). The skin membranes were soaked in Phosphate buffer pH 7.4 half an hour before using .The cleaned pig skin was used to separate the receiver and donor compartments of the FDC.

3.11.2 Formulation (F1) from section 3.10 was used for permeation study using pig skin mounted on a FDC, due to its acceptability, spreadability, and good permeation coefficient.

The diffusion parameters are determined as described under section 3.8, and compared to the results obtained by using natural chicken eggshell membrane.

3.12 Stability study of the selected Folic Acid gel (Formulation F1)

Folic acid gel formulation F1 was filled in Aluminum tubes, its final marketed package, to investigate of how the quality of an Active Pharmaceutical Ingredient (API) or Finished Pharmaceutical Product (FPP) varies with time under the influence of a variety of environmental factors such as temperature, humidity and light.

Environmental Conditions

Samples from the selected formula will be kept at different environmental conditions as given below for a 6 weeks and will be analyzed at initial, 3 weeks and 6 weeks.

Table (3.9): Stability Environmental Conditions

Study	Storage Condition	Testing time interval
Long Term	25°C ± 2°C/ 60% RH± 5% RH	initial, 2 weeks and 6 weeks
Intermediate	30°C ± 2°C/ 65% RH± 5% RH	initial, 2 weeks and 6 weeks
Accelerated	40°C ± 2°C/ 75RH± 5% RH	initial, 2 weeks and 6 weeks

Stability parameters

The gel Stability will be evaluated for the following parameters: Physical appearance, pH, and Drug content assay.

3.13 Analytical Test Methods

3.13.1 Description:

Place 2 g of gel on a white clean and dry paper then inspect it visually

3.13.2 Identification Test (HPLC):

In the assay method, the retention time of the major peak in the chromatogram of the test preparation corresponds to that in the chromatogram of the standard preparation.

3.13.3 Viscosity Testing:

Use rotary viscometer, equipped with spindle 96, rpm = 12. The viscosity is (6.5-7.5) x 10^4 cps measured at 20-25^{o}C.

3.13.4 pH measurement:

Mix 10g of gel with 90ml of distilled water. Use a suitable calibrated pH meter having combined glass electrode. Insert the electrode into the solution, and record the readings.

3.13.5 Assay:

Reference: based on test method of Folic acid injection as per USP 40.

Materials:

- Folic Acid Reference/ Working standard (RS/WS).
-Methanol HPLC grade.
-Potassium dihydrogen phosphate analytical grade.
- Ammonium Hydroxide
-Sodium Perchlorate
-Acetic Acid
-Phosphoric Acid
-Water HPLC grade.

Test Instruments & Equipment:

HPLC (Merck Hitachi) equipped with photodiode array UV detector.
Analytical Balance
Sonicator
Hot plate
Magnetic stirrer
1ml, 2ml, 3ml, 5 ml volumetric pipettes
25-ml, 100, 500, 1000 ml volumetric flasks

Preparation of Mobile Phase:

Transfer 35.1 g of sodium perchlorate and 1.40 g of monobasic potassium phosphate, accurately weighed, to a 1000-ml volumetric flask, add 7.0 ml of 1N potassium hydroxide, 40 ml of methanol, dilute with water to volume, and mix. Adjust with 1 N potassium hydroxide or phosphoric acid to a pH of 7.2.

Standard Preparation:

For Gel: Accurately weigh 25 mg Folic Acid Working standard, corrected for its water content, transfer to a 250-ml Volumetric flask, add 100 ml of phosphate buffer pH 7.4, sonicate, stir, and Complete Volume with phosphate buffer pH 7.4. Dilute 5 ml of this solution to 25 ml with phosphate buffer pH 7.4 and filter through 0.45 µm nylon filter.

For diffusion study: Prepare series of solutions with different concentrations to construct calibration curve:

Sr #	C mg/ml
1	0.0075
2	0.015
3	0.02
4	0.03

Sample Preparation:

- For Gel: A sample solution was prepared with a concentration equivalent to that in standard solution by transferring an accurately weighed portion of the sample equivalent to 2 mg of folic acid to a 100-ml volumetric flask, add 20 ml of phosphate buffer pH 7.4, sonicate, Stir, and Complete to Volume with phosphate buffer pH 7.4 and filter through 0.45 µm nylon filter.
- For diffusion samples: use the sample directly after filtration through 0.45 µm nylon filter.

Chromatographic Conditions:

- HPLC (Merck Elite) equipped with photodiode array UV detector.

Chromatographic System:

- Column: Octadecyl silane bonded to porous silica, 25 cm length and 4.6 mm I.d.
- Detection wave length: 254 nm.
- Flow rate: 1 ml/min.
- Injection Volume: 25 µL.

Procedure:

1-Separately inject equal volumes (about 20 µL) of both the standard preparation and the assay preparation into the Liquid chromatograph in triplicates.

-Record the chromatograms and measure the responses for the major peaks.

-Calculate the percentage of folic Acid in the product using the formula:

$$\% \text{ Folic Acid} = \frac{\text{Area of Assay Preparation peak}}{\text{Area of Standard Preparation Peak}} \times 100\%$$

2-To test the stability of Folic acid in Phosphate buffer pH 7.4: Keep the series of solution at room temperature for 24 hours and re-inject them into the Liquid chromatograph, record the chromatograms, measure the responses for the major peaks, and compare them with initial time.

Part 4

Results and Discussion

4.1 Analysis of Folic Acid:

Folic acid used in the study was analyzed according to BP test methods. Test results are shown in Table 4.1.

Table (4.1) Analysis results of Folic acid, BN# UT16030105 used in the study

Sr#	Test	Acceptance Criteria	Results
1	Content of Folic Acid	96.0-102.0% (on dried basis)	99.9
2	Characteristics	Yellowish orange micronized powder	Yellowish orange
3	Solubility	Practically insoluble in water and in most organic solvents. It dissolves in dilute acids and in alkaline solutions.	Practically insoluble in water, soluble in dilute acids and in 0.1N Sodium Hydroxide
4	Identification	A. Specific optical rotation: + 18º to + 22º (anhydrous substance). B. The principal peak in the chromatogram obtained with the test solution in assay is similar in retention time to the principal peak in the chromatogram obtained with reference solution	A. +21º B. Pass test
5	Water content	5.0% - 8.5%	7.6%
6	Sulfated ash	NMT 0.2%	<0.1%
7	Related Substances	NMT 0.5% of impurity A NMT 0.6% of impurity D NMT 0.5% of any other impurity NMT 1.0% of total impurities	0.1% Not detected <0.1% 0.5%
8	Particle Size	D90 should be less than 20 µm	10 µm

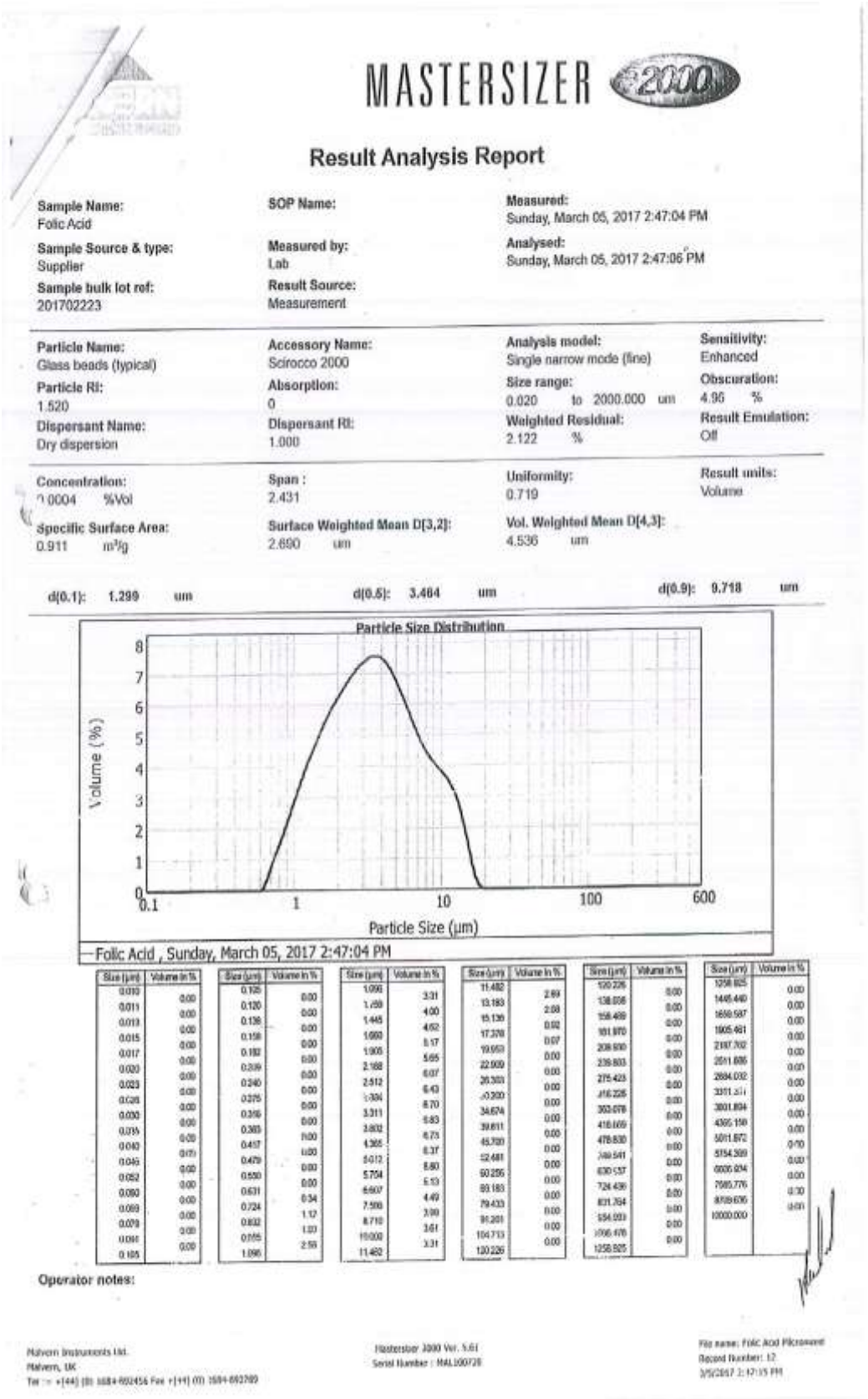


Figure 4-1: Results of particle size distribution for Folic acid used in the study

4.2. Solubility determination results

Saturated solutions of Folic acid were prepared at different pHs, their concentrations were determined by HPLC and measuring the responses at 254 nm (see section 3.13). Results are shown in table (4.2).

Table 4.2: The solubility results of Folic Acid in different solutions at 20C.

Folic acid in	Solubility g/100ml
Phosphate Buffer pH 7.4	0.680
Phosphate Buffer pH 6.8	0.300
Acetate Buffer pH 4.5	0.013
0.1% Triethanolamine	0.163
Water	0.00016

The solubility of Folic acid increases as the pH increases; hence there are two carboxylic groups in the molecule. The highest solubility was found when the pH of the solution was 7.4.

The solubility of Folic acid in water was found in literature to be 1.6 mg/L (Folic Acid, ChemIDplus database).

4.3. Studying the compatibility of Folic acid with excipients and penetration enhancers in aqueous solutions.

Different penetration enhancers and excipients were tested for their compatibility with Folic acid in water at different pH`s for a period of three days at 40 ºC; the results are shown in Table (4.3).

Table 4.3: The results of the compatibility study of Folic acid with excipients for three days at 40 ºC ± 2 ºC.

			% API	
Sr. #	Excipient	Concentration of excipient	**at Zero time**	after 3 days
1	Benzyl Alcohol	1%	100%	98.3
2	Carbopol 940	1%	100%	82.3
3	IPM	5%	100%	99.2
4	TEA	0.1%	100%	96.9
5	Tween 20	1%	100%	96.6
6	Propylene Glycol	10	100%	99.9
7	PSE-15	2%	100%	100
8	SLS	0.3%	100%	92.3
9	IPA	30%	100%	100.3

It is obvious that all excipients under study, except Carbopol are compatible with Folic acid. Carbopol 940 becomes gelatinous after 3 days at 40 ºC, which caused entrap of API.

4.4. Determination of best Penetration enhancer in Gel.

When n-octanol was used as soaking liquid, the eggshell membrane became corrugated, brittle and not suitable for the study. In the case of using polyamide membrane soaked in n-octanol, no penetration of Folic acid was observed during the first 36 hours of study. All diffusion results in this study are a result of using eggshell membrane soaked in IPM.

In this part the lag time (T_L) reflects the time required by API to pass through the intact membrane and reach to the receiver compartment. Diffusion coefficient (D) measures the membrane resistance encountered by a diffusant. Permeability coefficient (P) gives an indication about the distance passed by the substance within specific period. The partition coefficient (K) gives an indication about the ability of API to partition between the oily phase and aqueous phase, this parameter includes other diffusion parameters as shown in the calculation of diffusion parameters (part three). In the final part of this thesis we made attempts to compare the enhancement ratio (ER) of various penetration enhancers used. This is a ratio of permeability coefficients constants (P) following the use of penetration enhancer divided by the permeability coefficient before the use of penetration enhancer (P after / P before). The greater the ER, the greater the penetration enhancement ability of penetration enhancer used.

Table 4.4: A list of Folic acid gel formulae to be studied for permeability

Formula no.	Composition
F0	Folic acid 0.1% gel without Benzyl alcohol and PE
F1	Folic acid 0.1% gel with Benzyl alcohol and without PE
F2	Folic acid 0.1% gel with Benzyl alcohol and with 30% IPA
F3	Folic acid 0.1% gel with Benzyl alcohol and with 20% IPA
F4	Folic acid 0.1% gel with Benzyl alcohol and with 2% IPM

Formula of Folic acid 0.1% gel containing 5% IPM (F5), 1% Tween 20 (F6), 0.3% SLS (F7) and 2% PSE-15 (F8) were excluded from permeation study, because they became turbid and exhibited physical instability within two days at room temperature.

4.4.1. Formula no. F0, Folic acid 0.1% gel without Benzyl alcohol and PE

The basic Folic acid gel was prepared without using neither preservative (Benzyl alcohol) nor penetration enhancer as a control formulation to constitute a base for comparison.

Samples were taken every one hour from the receiver compartment and analyzed by HPLC for the amount of Folic acid.

Table (4.5) illustrates the assay results of API penetrated to the receiving compartment by time. Area under peaks (Area1, Area2, Area3) was presented in triplicates, and the cumulative amount of drug penetrated (Q) per unit of membrane area was determined and plotted as a function of time Fig. (4-2). The linear part of the curve was plotted in Fig. (4.2) and the diffusion parameters were calculated for API alone and tabulated in Table (4.7). The T_L was calculated by dividing the intercept of the equation of flux profile on the slope from the same equation. The diffusion parameter (D) was calculated using equation 11:

$D = h^2/6.T_L$ …….……..…. (11)

The permeability coefficient (P) was calculated by dividing the value of the slope of the flux profile by the concentration of API in the donor compartment (1mg/ml). The permeability coefficient (P) was used here as the main value in the comparison between the activity of different penetration enhancers, since its value was obtained from all diffusion parameters as shown in equation 9.

$K = (P. h) / D$ …….……. (9)

.

.

Table 4.5: Data obtained from the diffusion of Formula no. F0, through eggshell membrane using Franz diffusion cell, with neither preservative nor penetration enhancer

Time [h]	Area 1	Area 2	Area 3	Mean Area	SD	RSD%	Concentration of sample (mg/ml)	Calculated amount released = C*V [mg]	Q: cumulative amount released [mg]	m: cumulative amount released/ cm^2 (mg/cm^2)
1	699561	872269	923255	831695	117236	14	0.006192487	0.046443652	0.046443652	0.026239352
2	1055756	1260028	1258295	1191359	117439	9.8	0.008956394	0.067172958	0.073365445	0.041449404
3	1265495	1425770	1530199	1407154	133330	9.47	0.010614713	0.079610345	0.094759227	0.053536286
4	1434761	1570041	1675903	1560235	120869	7.74	0.011791093	0.088433199	0.114196793	0.064517962
5	1500503	1583096	1767895	1617165	136912	8.46	0.012228583	0.091714371	0.129269058	0.073033366
6	1506241	1563875	1793811	1621309	152145	9.38	0.012260428	0.091953211	0.141736481	0.080077108
7	1530304	1606708	1854080	1663698	169244	10.1	0.012586175	0.09439631	0.156440008	0.088384185

Table 4.2 shows the analysis results of Folic acid, this diffusion study was done three times. The cumulative amount of Folic acid per unit of membrane area was calculated and plotted as a function of time. See figure (4.2).

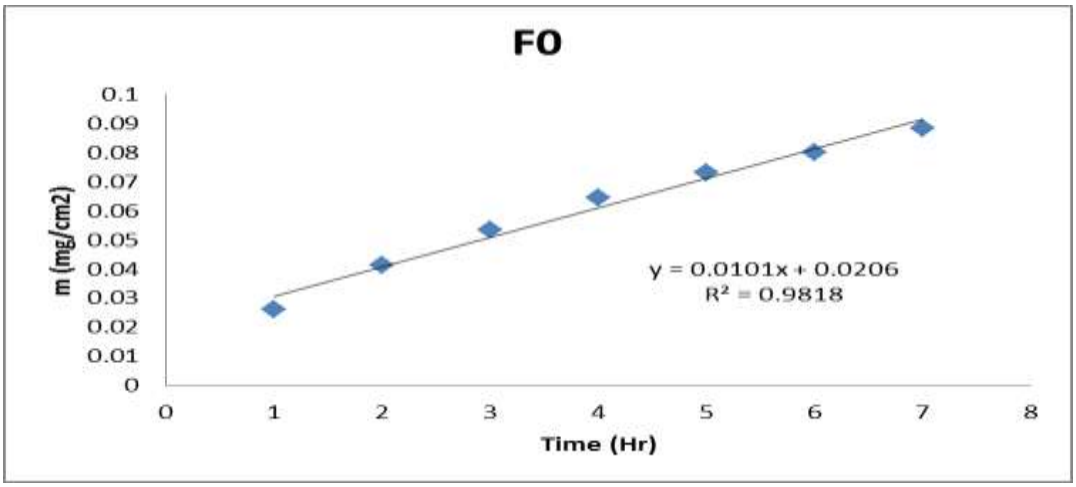


Figure 4.2: In vitro permeation profile for the cumulative amount of Folic acid penetrated per unit area of eggshell membrane (mg/cm²) for Formula no. F0.

The best linear line is determined on figure (4.2) by Excel 2007, from which the linear line equation is determined. This equation helps in determining the slope and the x intercept; these are used for further calculation of diffusion parameters. The diffusion parameters were calculated according to table (3.7). The diffusion parameters are shown in table (4.6).

Table 4.6: Diffusion parameters for Formula no. F0, no penetration enhancer introduced.

Formulation	Slope	Intercept	T_L	D	P	K	ER
F0	0.0101	0.0206	2.039604 h	3.26861E-05	0.0101	6.18	1.0000

The permeability parameter of this basic gel formula was used as a reference for penetration enhancement comparison**.**

Folic acid showed some penetration through the natural eggshell membrane due to its solubility in hydrophilic and hydrophobic parts of the membrane.

4.4.2. Formula no. F1, Folic acid 0.1% gel with 1%t Benzyl alcohol and without PE

Benzyl alcohol is an antimicrobial preservative used in cosmetics, foods, and a wide range of pharmaceutical formulations including oral and parenteral preparations, at concentrations up to 2.0% v/v. The typical concentration used is 1% v/v.(Croshaw B.,1977).

Gel of Folic acid 0.1% and benzyl alcohol 1% was prepared according to the general method described in section 3.6.2. The permeability results of drug through the natural eggshell membrane are shown in table (4.7) and Fig. (4.3).

The cumulative amount of API permeated through unit area of membrane was then calculated as mentioned before, and the linear section, i.e. the steady state flux was plotted versus time Fig. (4.3).

Table 4.7: Data obtained from the diffusion of Formula no. F1, through eggshell membrane using Franz diffusion cell, with 1% benzyl alcohol as preservative, and without penetration enhancer

Time [h]	Area 1	Area 2	Area 3	Mean Area	SD	RSD%	Concentration of sample (mg/ml)	Calculated amount released = C*V [mg]	Q: cumulative amount released [mg]	m: cumulative amount released/ cm^2 (mg/cm^2)
1	2962505	3304734	3435623	3234287	244299	7.5	0.018980486	0.142353642	0.142353642	0.080425786
2	4564978	4618995	5023566	4735846	250632	5.29	0.027619248	0.207144358	0.226124844	0.127754149
3	5766635	5733431	6044888	5848318	171042	2.9	0.034019516	0.255146373	0.301746106	0.170478026
4	6048765	6334950	6583448	6322387	267562	4.2	0.036947568	0.27560676	0.35622601	0.201252378
5	6674837	7020502	7001786	6864793	175358	2.55	0.039006453	0.299483982	0.4163508	0.235251865
6	7400251	7845440	7482482	7576057	236887	3.1	0.043959536	0.329696522	0.488327871	0.275891452
7	7144112	7365030	7098791	7202644	142444	1.97	0.041811218	0.313584138	0.516175022	0.291624306

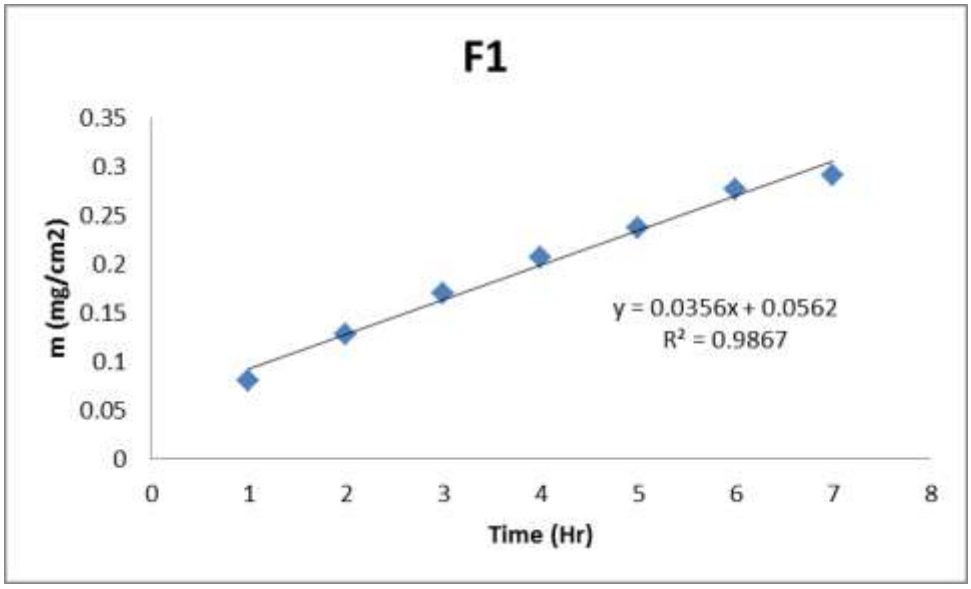


Figure 4.3: In vitro permeation profile for the cumulative amount of Folic acid penetrated per unit area of eggshell membrane (mg/cm²) for Formula no. **F1**, with 1% benzyl alcohol as preservative, and without penetration enhancer

The diffusion parameters were calculated according to table (4.7) and the enhancement ratio is determined. (See table 4.8).

Table 4.8: Diffusion parameters for Formula no F1 with 1% benzyl alcohol as preservative, and without penetration enhancer

Formulation	Slope	Intercept	T_L	D	P	K	ER
F1	0.0356	0.0562	1.578652	4.22301E-05	0.0356	16.86	3.524752475

The rate of diffusion of API in the presence of Benzyl alcohol is faster than when it is alone, this is indicated by the value of high permeability coefficient (P) from which the enhancement ratio (ER) is found to be (3.52). It is obvious that Benzyl alcohol is not working only as preservative, but it is also penetration enhancer.
This great value of permeation coefficient of Benzyl alcohol is probably due to its ability to extract lipids and proteins and thereby increase the porosity of the

stratum corneum by removing some parts of oil through which the API can shuttle. (Williams, A, Barry, B. 2004).

4.4.3 Formula no. F2, Folic acid 0.1% gel with 1%t Benzyl alcohol and 30% IPA
Isopropyl alcohol is used in cosmetics and pharmaceutical formulations, primarily as a solvent in topical formulations (Rafiee Tehrani H, Mehramizi A., 2000). Its enhancing effect on skin penetration occurs by increasing the permeability of *Stratum corneum* (Goto S, Uchida T,1993).
Using IPA 20% as potential penetration enhancer and following the same general procedure, the data measured are shown in table (4.9).

Table 4.9: Data obtained from the diffusion of Formula no. F2, through eggshell membrane using Franz diffusion cell, with 1% benzyl alcohol as preservative, and 30% IPA as potential penetration enhancer

Time [h]	Area 1	Area 2	Area 3	Mean Area	SD	RSD%	Concentration of sample (mg/ml)	Calculated amount released = C*V [mg]	Q: cumulative amount released [mg]	m: cumulative amount released/ cm^2 (mg/cm^2)
1	371878	353074	407263	377405	27514	7.29	0.002701408	0.020260559	0.020260559	0.011446643
2	591051	618091	665491	624877	3768	6	0.004603159	0.034523695	0.037225102	0.021031131
3	775983	811146	875007	820712	50200	6.4	0.006108086	0.045810645	0.053115212	0.030008594
4	809493	878818	908805	865705	50937	5.88	0.006453846	0.048403845	0.061816498	0.034924575
5	868103	……	993648	930875	88773	9.5	0.006954659	0.05215994	0.072026439	0.040692903
6	917009	1003409	1051630	990682	990682	6.88	0.007414258	0.055606938	0.082428096	0.046569546
7	915837	1026677	1080221	1007578	83839	8.3	0.007544096	0.056580723	0.090816139	0.051308553

In vitro permeation profile for the cumulative amount of Folic acid penetrated per unit area for Formula no F2 is shown in figure (4.4); the Steady state diagram is shown in figure (4.4)

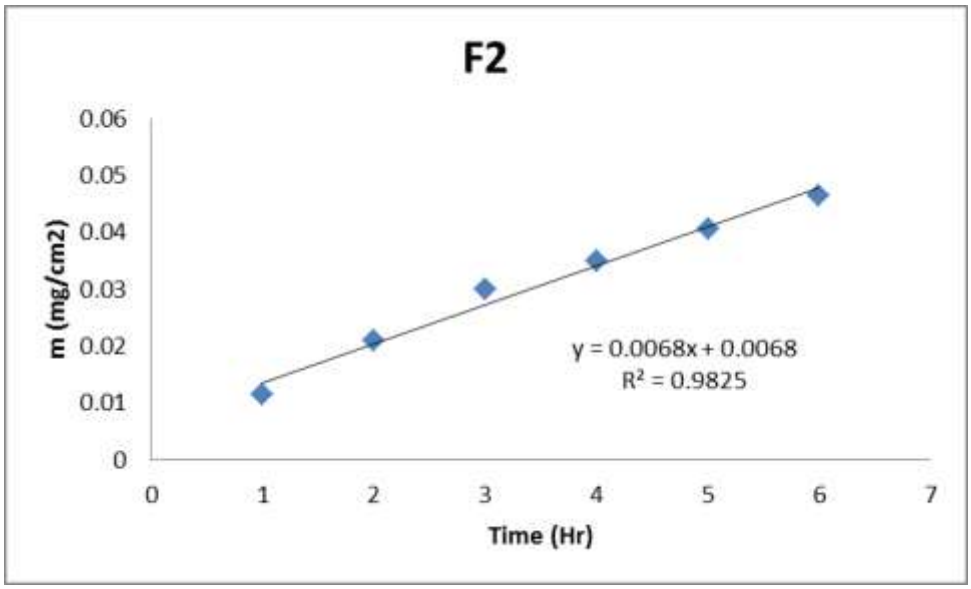


Figure 4.4: In vitro permeation profile for the cumulative amount of Folic acid penetrated per unit area of eggshell membrane (mg/cm²) for Formula no.F2, using 30% IPA as penetration enhancer

The diffusion parameters were calculated according to table (4.9) and the enhancement ratio is determined. (See table 4.10).

Table 4.10: Diffusion parameters for Formula no F2 with 1% benzyl alcohol as preservative, and 30% IPA as penetration enhancer

Formulation	Slope	Intercept	T_L	D	P	K	ER
F2	0.0068	0.0068	1	6.66667E-05	0.0068	2.04	0.191011236

The calculated enhancement ratio was reduced to 20% relative to control formulation (F0), and to 6% relative to formulation with benzyl alcohol (F1). This may be due to the fact that Folic acid is practically insoluble in most organic

solvents; the addition of IPA reduced the solubility of Folic acid in gel dosage form, and consequently works as penetration retardant. Also pH change may affect the diffusion rate.

4.4.4 Formula no. F3, Folic acid 0.1% gel with 1%t Benzyl alcohol and 20% IPA

In this formulation the percentage of IPA was reduced to constitute 20% of formula.The data of HPLC analysis of Formula no. F3 is shown in table (4.11). The cumulative amount of Folic acid penetrated per unit area is shown in figure (4.5).

The calculated diffusion parameters are presented in table (4.12). The enhancement ratio of 20% IPA was found to be 2.84.

Table 4.11: Data obtained from the diffusion of Formula no. F3, through eggshell membrane using Franz diffusion cell, with 1% benzyl alcohol as preservative, and 20% IPA as potential penetration enhancer

Time [h]	Area 1	Area 2	Area 3	Mean Area	SD	RSD%	Concentration of sample (mg/ml)	Calculated amount released = C*V [mg]	Q: cumulative amount released [mg]	m: cumulative amount released/ cm^2 (mg/cm^2)
1	228	631	301	387	214	55.5	0.001686638	0.012649784	0.012649784	0.00714677
2	2678	2513	2512	2568	95	3.7	0.011088226	0.083161693	0.08484833	0.04793691
3	3599	3019	3213	3277	295	9	0.014142126	0.106065943	0.118840806	0.067141699
4	3838	3561	3636	3678	143	3.89	0.015871616	0.119037118	0.145954107	0.082459947
5	4076	3745	3832	3884	171	4.4	0.016759344	0.125695079	0.168483684	0.095188522
6	4399	4119	4113	4211	163	3.88	0.018165632	0.136242239	0.195790188	0.110615925

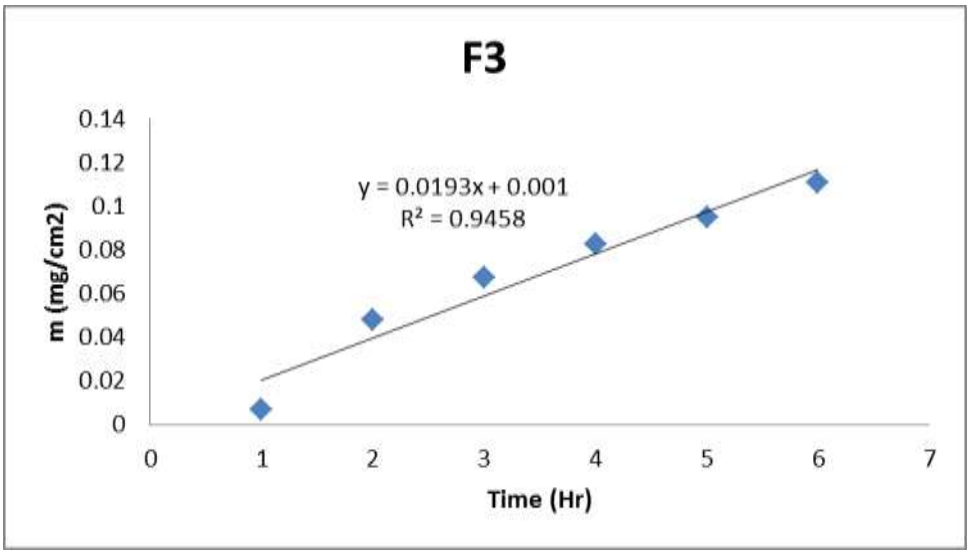


Figure 4.5: In vitro permeation profile for the cumulative amount of Folic acid penetrated per unit area of eggshell membrane (mg/cm²) for Formula no.F3, using 20% IPA as penetration enhancer

Table 4.12: Diffusion parameters for Formula no F3 with 1% benzyl alcohol as preservative, and 20% IPA as penetration enhancer

Formulation	Slope	Intercept	T_L	D	P	K	ER
F3	0.0193	0.001	0.051813	0.001286667	0.0193	0.3	2.838235294

4.4.5 Formula no. F4, Folic acid 0.1% gel with 1% Benzyl alcohol and 2% IPM

Isopropyl Myristate (IPM) is a non-greasy emollient that is absorbed readily by the skin. It is used as a component of semisolid bases and as a solvent for many substances applied topically. Using IPM 2% as potential penetration enhancer and following the same general procedure, the data measured are shown in table (4.13).

The cumulative amount of Folic acid penetrated per unit area is shown in figure (4.6).
The calculated diffusion parameters are presented in table (4.14).

Table 4.13: Data obtained from the diffusion of Formula no. F4, through eggshell membrane using Franz diffusion cell, with 1% benzyl alcohol as preservative, and 2% IPM as potential penetration enhancer

Time [h]	Area 1	Area 2	Area 3	Mean Area	SD	RSD%	Concentration of sample (mg/ml)	Calculated amount released = C*V [mg]	Q: cumulative amount released [mg]	m: cumulative amount released/ cm2 (mg/cm2)
1	1983507	1511557	1515263	1670109	271416	16.2	0.012635441	0.094765809	0.094765809	0.053540005
2	2651130	2067666	2111096	2276631	325052	14.2	0.017296377	0.129722824	0.142358265	0.080428398
3	3154304	2428066	2556624	2712998	387550	14.2	0.020649723	0.154872922	0.18480474	0.104409458
4	3334832	2594653	2687711	2872399	403172	14	0.021874671	0.16406003	0.214641571	0.121266424
5	3580544	2899390	2844571	3108168	410006	13	0.023686483	0.177648623	0.250104835	0.141302166
6	3480193	2934008	2968120	3127440	305968	9.7	0.023834582	0.178759369	0.274902063	0.1553119
7	3621372	3166364	3136773	3308169	271644	8.2	0.025223429	0.18917572	0.309152997	0.17466271

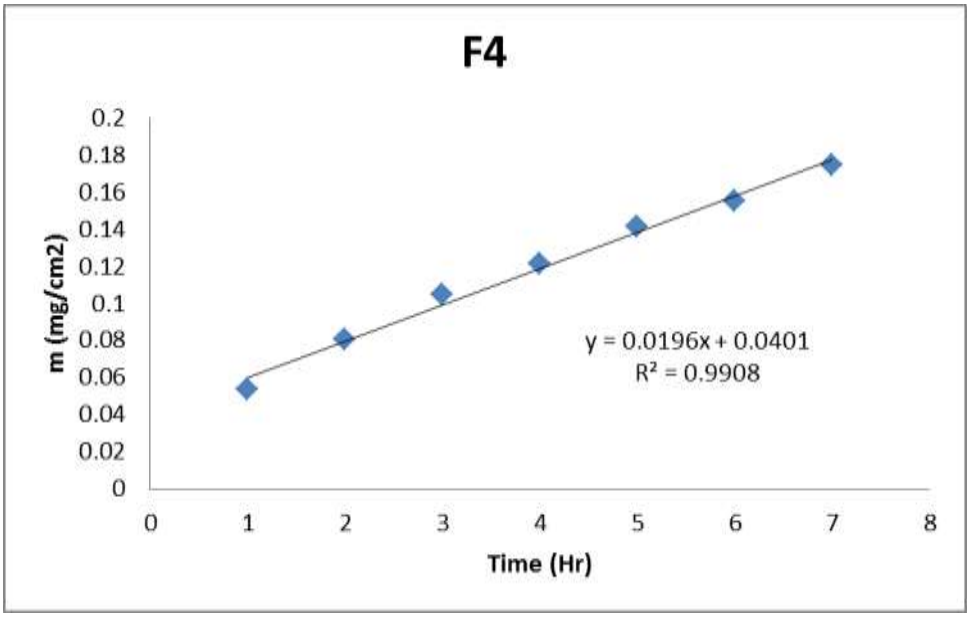


Figure 4.6: In vitro permeation profile for the cumulative amount of Folic acid penetrated per unit area of eggshell membrane (mg/cm²) for Formula no.F4, using 2% IPM as penetration enhancer

.

Table: 4.14: Diffusion parameters for Formula no F4 with 1% benzyl alcohol as preservative, and 2% IPM as penetration enhancer

Formulation	Slope	Intercept	T_L	D	P	K	ER
F4	0.0196	0.0401	2.045918	3.25852E-05	0.0196	12.03	1.015544041

The presence of IPM in 2% has neglectable penetration enhancement. At higher concentration it may exhibit significant penetration enhancement.

4.5 Selecting the best penetration enhancer

Table 4.15 summarizes the diffusion parameters of all formulations tested.

Table 4.15: summary of diffusion parameters for different formulations of Folic acid gel 0.1%

Formulation	Slope	Intercept	T_L	D	P	K	ER
F0	0.0101	0.0206	2.039604 h	3.26861E-05	0.0101	6.18	1.0000
F1	0.0356	0.0562	1.578652	4.22301E-05	0.0356	16.86	3.524752475
F2	0.0068	0.0068	1.00	6.66667E-05	0.0068	2.04	0.191011236
F3	0.0193	0.001	0.051813	0.001286667	0.0193	0.3	2.838235294
F4	0.0196	0.0401	2.045918	3.25852E-05	0.0196	12.03	1.015544041

It is obvious from the above table that the best penetration enhancement was found for formulation no **F1**), when benzyl alcohol alone was used as preservative as well as penetration enhancer. Formulation F1 exhibited ER about 3.5 times relative to control formulation.

4.6. Pig Skin Permeation results

As mentioned in section 4.5, the best penetration enhancement was found for formulation F1. This formulation was scaled up and tested for permeation through pig skin using the same configuration of FDC apparatus. Thickness of Pig skin was found to be 1.3cm. The sampling points were after 2 hours from the start of the test, and then every hour till 10 h., and finally after 24 h. The gel formula used was gel formula **F1**:

The gel specifications and test results are shown in table (4.16), and the permeation data obtained are shown in table (4.17)

Table 4.16: Specifications and test results of final gel formula (no.F1) that was tested for permeation through Pig skin

Specification	Description	Test results
Color	Yellow	Yellow
pH	6.0-7.0	6.4
Viscosity	(6.5-7.5) x 10^4 cps at 20-25ºC.	7.4 x10^4
Spreadability	Easily spreadable	Easily spreadable on the skin
Label Claim: Folic acid	0.1% w/w	0.1%
Content of Folic Acid	90.0 – 110.0% of label claim	102.2%

Table 4.17: Data obtained from the diffusion of Formula no. F1, containing benzyl alcohol 1% as preservative and penetration enhancer through Pig Skin using Franz diffusion cell

Time [h]	Area 1	Area 2	Area 3	Mean Area	SD	RSD %	Concentration of sample (mg/ml)	Calculated amount released = C*V [mg]	Q: cumulative amount released [mg]	m: cumulative amount released/ cm^2 (mg/cm^2)
2	7171	7115	18974	11087	6830	61.6	2.67E-05	0.000200097	0.000200097	0.000113049
3	30715	10897	71399	37670	30844	81.8	9.06486E-05	0.000679865	0.000706544	0.000399178
4	73226	34507	158664	88799	63526	71.5	0.000483559	0.003626695	0.003744023	0.002115267
5	116977	55667	249978	140874	99335	70	0.00088374	0.006628048	0.007228935	0.004084144
6	189130	99242	330865	206412	116774	56	0.001387379	0.010405343	0.011889971	0.006717497
7	261088	153306	465060	293151	158330	54	0.002053942	0.015404563	0.018276569	0.010325745
8	353586	216426	601123	390378	194969	49	0.002801101	0.02100826	0.025934208	0.014652095
9	452889	290125	754593	499202	235672	47	0.00363738	0.027280353	0.034890074	0.019711906
10	562856	393249	885934	614013	250294	40	0.004519668	0.033897507	0.04466105	0.025232231
24	2538191	2263047	3513026	2771421	656817	23.7	0.021098686	0.158240143	0.173523353	0.098035793

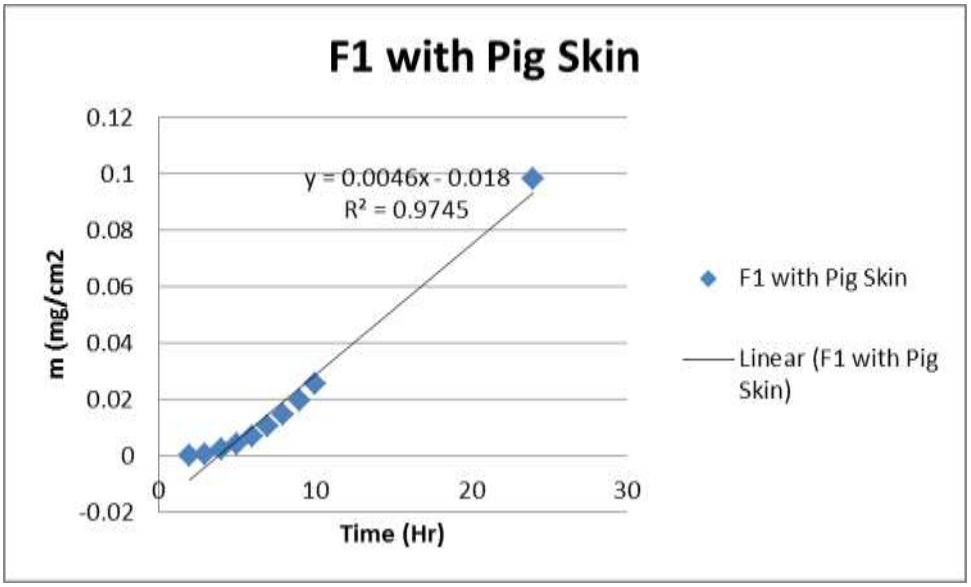


Figure 4.7: In vitro permeation profile for the cumulative amount of **Formula no. F1,** containing benzyl alcohol 1% as preservative and penetration enhancer through Pig Skin using Franz diffusion cell

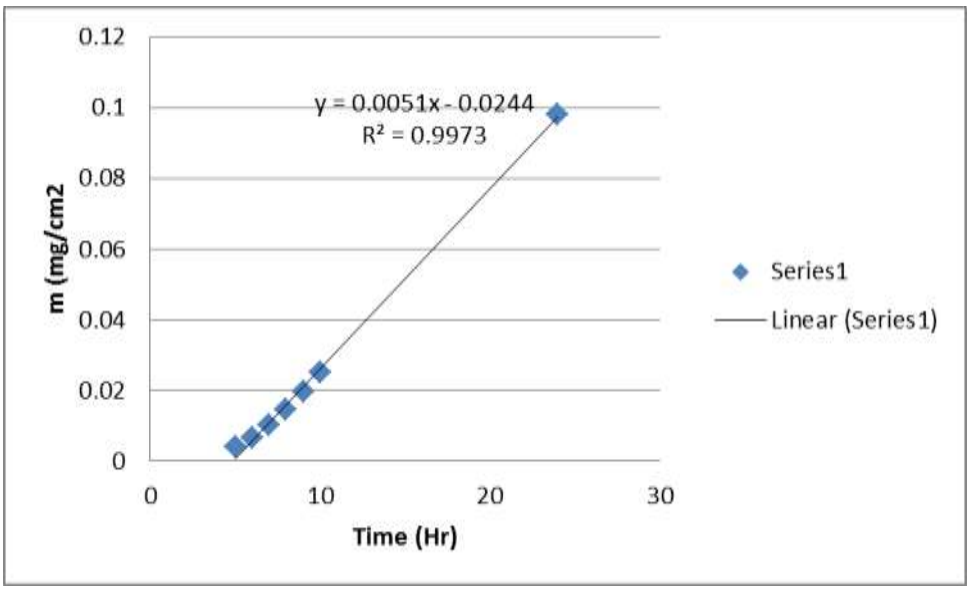


Figure 4.8: In vitro steady state flux diagram of **Formula no. F1,** containing benzyl alcohol 1% as preservative and penetration enhancer through Pig Skin using Franz diffusion cell

From the steady state flux diagram of **Formula no. F1,** the diffusion parameters were calculated.

Table 4.18: Diffusion parameters for Formula no F1, containing benzyl alcohol 1% as preservative and penetration enhancer through Pig Skin using Franz diffusion cell

Formulation	Slope	Intercept	T_L	D	P	K	
F1	**0.0051**	**0.0244**	**4.7843 h**	**0.00058873**	**0.005**	**1.102**	

Table 4.19 shows the Comparison of Diffusion parameters for **Formula no F1,** between eggshell membrane and Pig Skin.

Table 4.19: Comparison of Diffusion parameters for Formula no F1, containing benzyl alcohol 1% as preservative and penetration enhancer through eggshell membrane and Pig Skin using Franz diffusion cell

	Eggshell membrane	Pig Skin
$\mathbf{T_L}$ [h]	1.58 h	4.78
D [cm^2. h^{-1}]	4.22 $x10^{-5}$	5.89 $x10^{-4}$
P	0.036	**0.005**
K	16.9	1.1

4.7 Stability results of Formulation F1.

Formulation F1, was stored at different storage conditions, at long term condition ($25^oC \pm 2^oC$/ 60% RH± 5% RH), at intermediate conditions ($30^oC \pm 2^oC$/ 65%RH± 5%RH) and at accelerated conditions ($40^oC \pm 2^oC$ / 75RH± 5%RH), the stability samples were analyzed at zero time, 14 days and 42 days as schedule in the methodology stability section.

The stability results are tabulated in Tables (4.20-22)

Table 4.20: Stability results of FA 0.1% gel (F1) @ 25°C ± 2°C/ 60% RH± 5% RH

Characteristics	Acceptance Criteria	@ initial time	After 14 days	After 42 days
Appearance	Yellow homogeneous gel	Conform	Conform	Conform
pH	6.0-7.0	6.4	6.3	6.4
Spreadability	Easily spreadable on the skin	Easily spreadable	Easily spreadable	Easily spreadable
Viscosity	(6.5-7.5) x 10^4 cps at 20-25°C.	7.4 x10^4	7.2x10^4	7.5x10^4
Content of Folic Acid	90.0 – 110.0% of label claim	102.0%	103.9%	99.9%

Table 4.21: Stability results of FA 0.1% gel (F1) @ 30°C ± 2°C/ 65% RH± 5% RH

Characteristics	Acceptance Criteria	@ initial time	After 14 days	After 42 days
Appearance	Yellow homogeneous gel	Conform	Conform	Conform
pH	6.0-7.0	6.4	6.2	6.3
Spreadability	Easily spreadable on the skin	Easily spreadable	Easily spreadable	Easily spreadable
Viscosity	(6.5-7.5) x 10^4 cps at 20-25°C.	7.4 x10^4	7.4x10^4	7.5x10^4
Content of Folic Acid	90.0 – 110.0% of label claim	102.0%	97.5%	98.8%

Table 4.22: Stability results of FA 0.1% gel (F1) @ 40°C ± 2°C/ 75% RH± 5% RH

Characteristics	Acceptance Criteria	@ initial time	After 14 days	After 42 days
Appearance	Yellow homogeneous gel	Conform	Conform	Conform
pH	6.0-7.0	6.4	6.5	6.5
Spreadability	Easily spreadable on the skin	Easily spreadable	Easily spreadable	Easily spreadable
Viscosity	(6.5-7.5) x 10^4 cps at 20-25°C.	7.4 x10^4	7.1x10^4	6.7x10^4
Content of Folic Acid	90.0 – 110.0% of label claim	102.0%	99.1%	97.4%

Part Five

Conclusion

Conclusion

In the present work, the solubility of Folic acid was determined in different solutions and was found to increase as the pH increases.

Compatibility study of Folic acid with different excipients was carried out; almost all excipients used in this study were found to be compatible with Folic acid except Carbopol that caused precipitation due to its acidic characters.

The influence of selected penetration enhancers included in a 0.1 % w/w Folic acid gel preparation was investigated through natural eggshell membrane impregnated in Isopropyl Myristate. The enhancement ratio was calculated for each penetration enhancer and found to be in the following order:

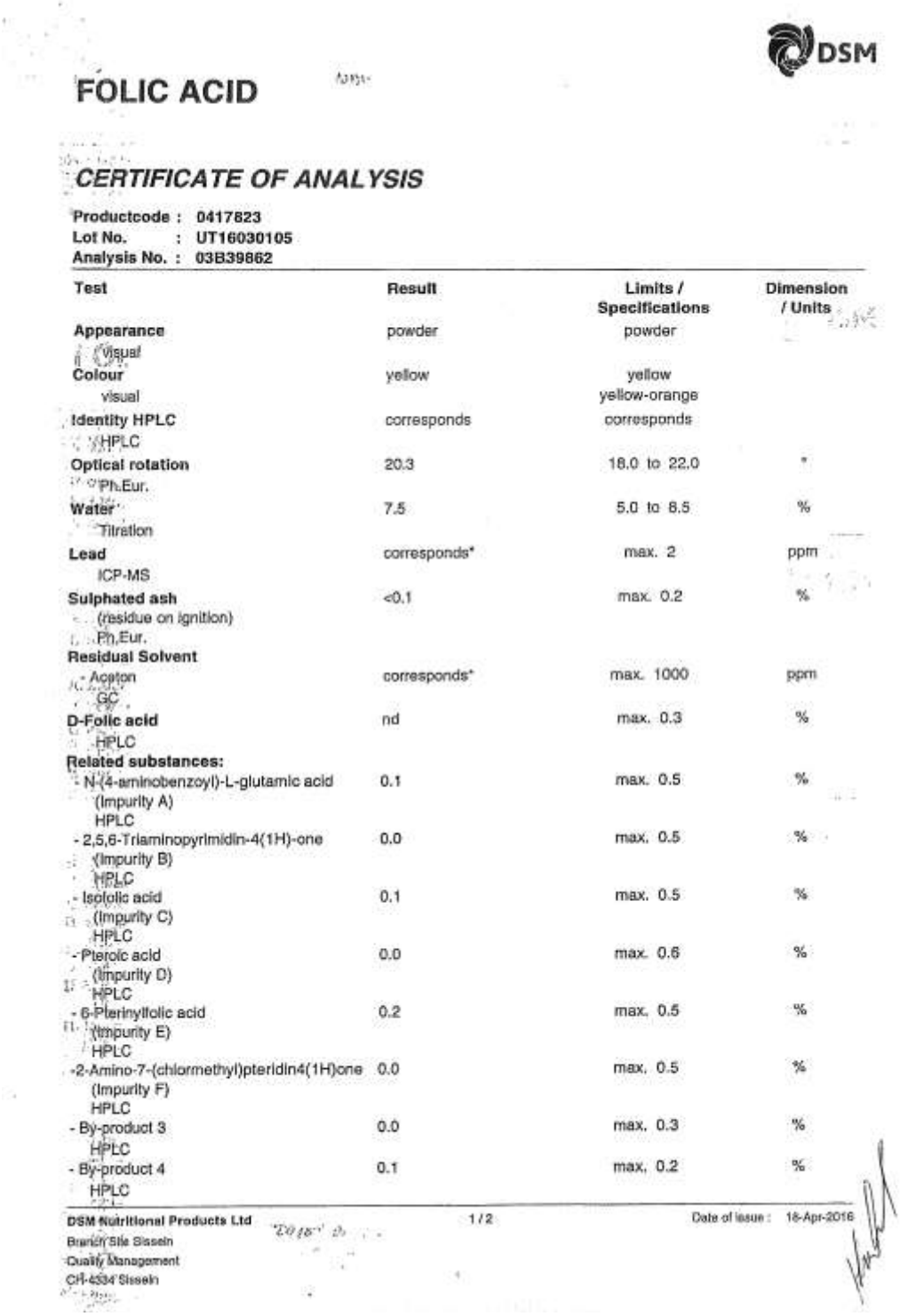
Benzyl alcohol > Benzyl alcohol + 20% IPA > Benzyl alcohol + 2% IPM> Benzyl alcohol + 30% IPA

Topical 0.1% Folic acid gel was formulated with the selected penetration enhancer Benzyl alcohol, and the permeation was investigated through 1.3 mm thickness pig skin. A comparison between the diffusion parameters was made between using natural eggshell membrane and pig skin. The lag time was found to be three folds in the case of pig skin relative to eggshell membrane, and the diffusion coefficient was about fourteen folds in the case of pig skin relative to eggshell membrane, while the permeability coefficient was seven folds in the case of eggshell membrane relative to pig skin.

The natural eggshell membrane showed better permeation results than pig skin, suggesting that the pig skin is more complex than eggshell bio-membrane and the difference between their thicknesses.

Part Six

Appendix



DSM

FOLIC ACID

CERTIFICATE OF ANALYSIS

Productcode : 0417823
Lot No. : UT16030105
Analysis No. : 03B39862

Test	Result	Limits / Specifications	Dimension / Units
Appearance visual	powder	powder	
Colour visual	yellow	yellow yellow-orange	
Identity HPLC HPLC	corresponds	corresponds	
Optical rotation Ph.Eur.	20.3	18.0 to 22.0	°
Water Titration	7.5	5.0 to 8.5	%
Lead ICP-MS	corresponds*	max. 2	ppm
Sulphated ash (residue on ignition) Ph.Eur.	<0.1	max. 0.2	%
Residual Solvent			
- Aceton GC	corresponds*	max. 1000	ppm
D-Folic acid HPLC	nd	max. 0.3	%
Related substances:			
- N-(4-aminobenzoyl)-L-glutamic acid (Impurity A) HPLC	0.1	max. 0.5	%
- 2,5,6-Triaminopyrimidin-4(1H)-one (Impurity B) HPLC	0.0	max. 0.5	%
- Isofolic acid (Impurity C) HPLC	0.1	max. 0.5	%
- Pteroic acid (Impurity D) HPLC	0.0	max. 0.6	%
- 6-Pterinylfolic acid (Impurity E) HPLC	0.2	max. 0.5	%
-2-Amino-7-(chlormethyl)pteridin4(1H)one (Impurity F) HPLC	0.0	max. 0.5	%
- By-product 3 HPLC	0.0	max. 0.3	%
- By-product 4 HPLC	0.1	max. 0.2	%

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Quality Management
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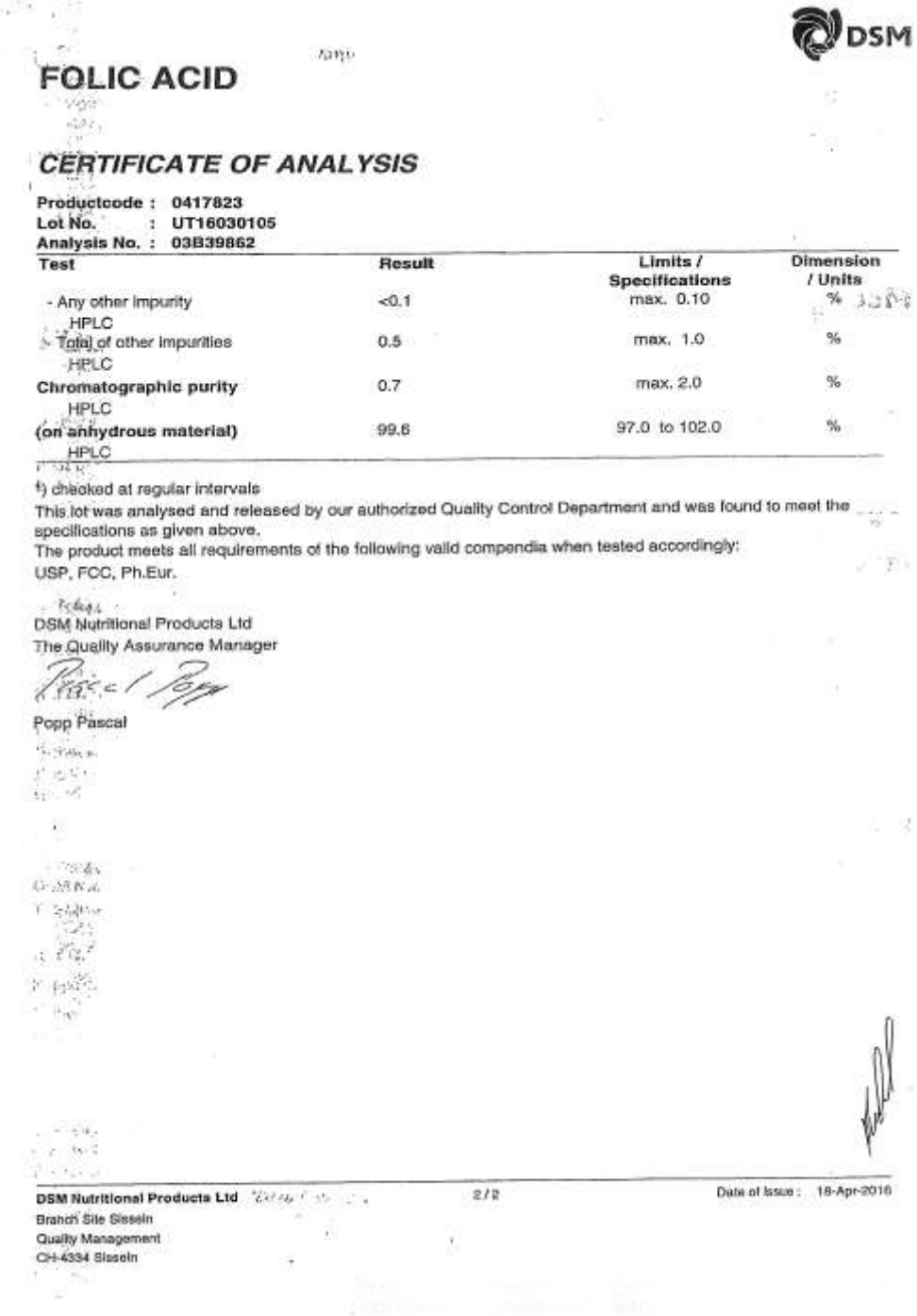
Date of issue : 18-Apr-2016

Figure 5.1: COA of Folic acid used in the study from the manufacturer DSM page 1/2



FOLIC ACID

CERTIFICATE OF ANALYSIS

Productcode : 0417823
Lot No. : UT16030105
Analysis No. : 03B39862

Test	Result	Limits / Specifications	Dimension / Units
- Any other impurity HPLC	<0.1	max. 0.10	%
- Total of other impurities HPLC	0.5	max. 1.0	%
Chromatographic purity HPLC	0.7	max. 2.0	%
(on anhydrous material) HPLC	99.6	97.0 to 102.0	%

1) checked at regular intervals

This lot was analysed and released by our authorized Quality Control Department and was found to meet the specifications as given above.
The product meets all requirements of the following valid compendia when tested accordingly:
USP, FCC, Ph.Eur.

DSM Nutritional Products Ltd
The Quality Assurance Manager

Popp Pascal

DSM Nutritional Products Ltd
Branch Site Sisseln
Quality Management
CH-4334 Sisseln

2 / 2

Date of Issue : 18-Apr-2016

Figure 5.2: COA of Folic acid used in the study from the manufacturer DSM page 2/2

Empower 3

Jerusalem Pharmaceutical

SAMPLE INFORMATION

Sample Name:	Folic Acid Std 0.015mg/ml	Acquired By:	System
Sample Type:	Unknown	Sample Set Name	Folic acid 040418
Vial:	2	Acq. Method Set:	Folic acid
Injection #:	2	Processing Method	Folic acid
Injection Volume:	25.00 ul	Channel Name:	2998 Ch1 254nm@1.2nm
Run Time:	4.0 Minutes	Proc. Chnl. Descr.:	2998 Ch1 254nm@1.2nm
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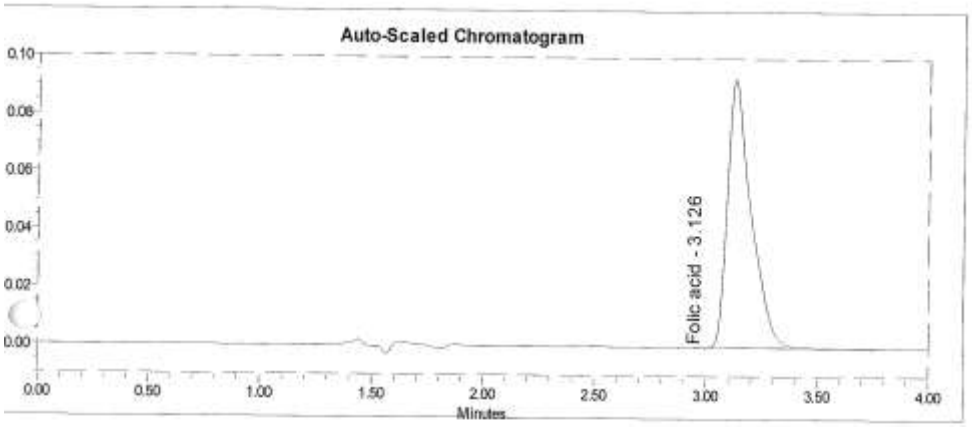
Folic Acid Solubility



kResults

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Folic acid	3.126	691850	93136	45.400	

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Report Method: Jerusalem Pharmaceutica
Report Method ID 50474

Project Name: Validation
Date Printed: 4/5/2018

Figure 5.3: A chromatograph of Folic acid reference standard using Waters HPLC

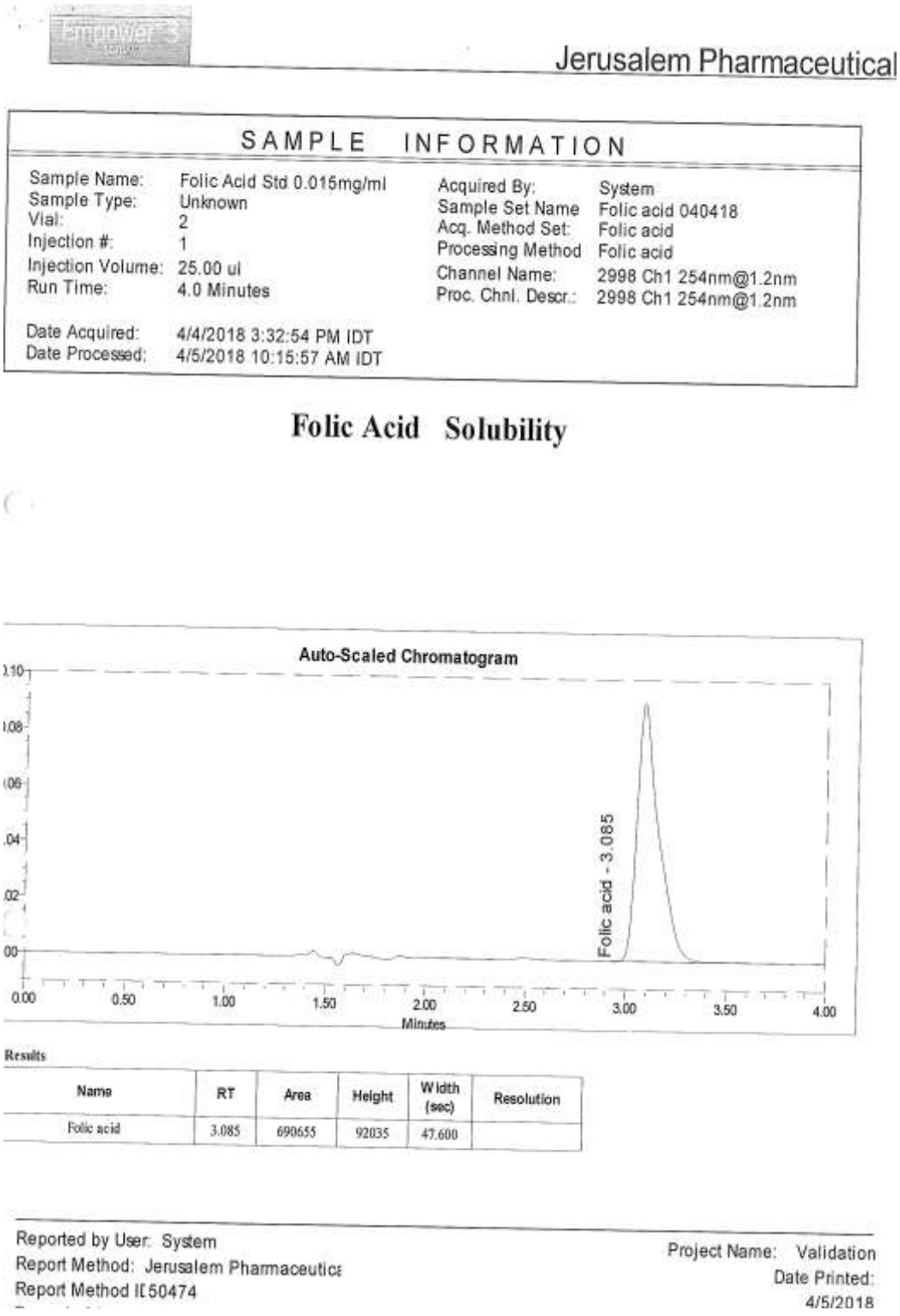

Figure 5.4: A chromatograph of Folic acid reference standard using Waters HPLC

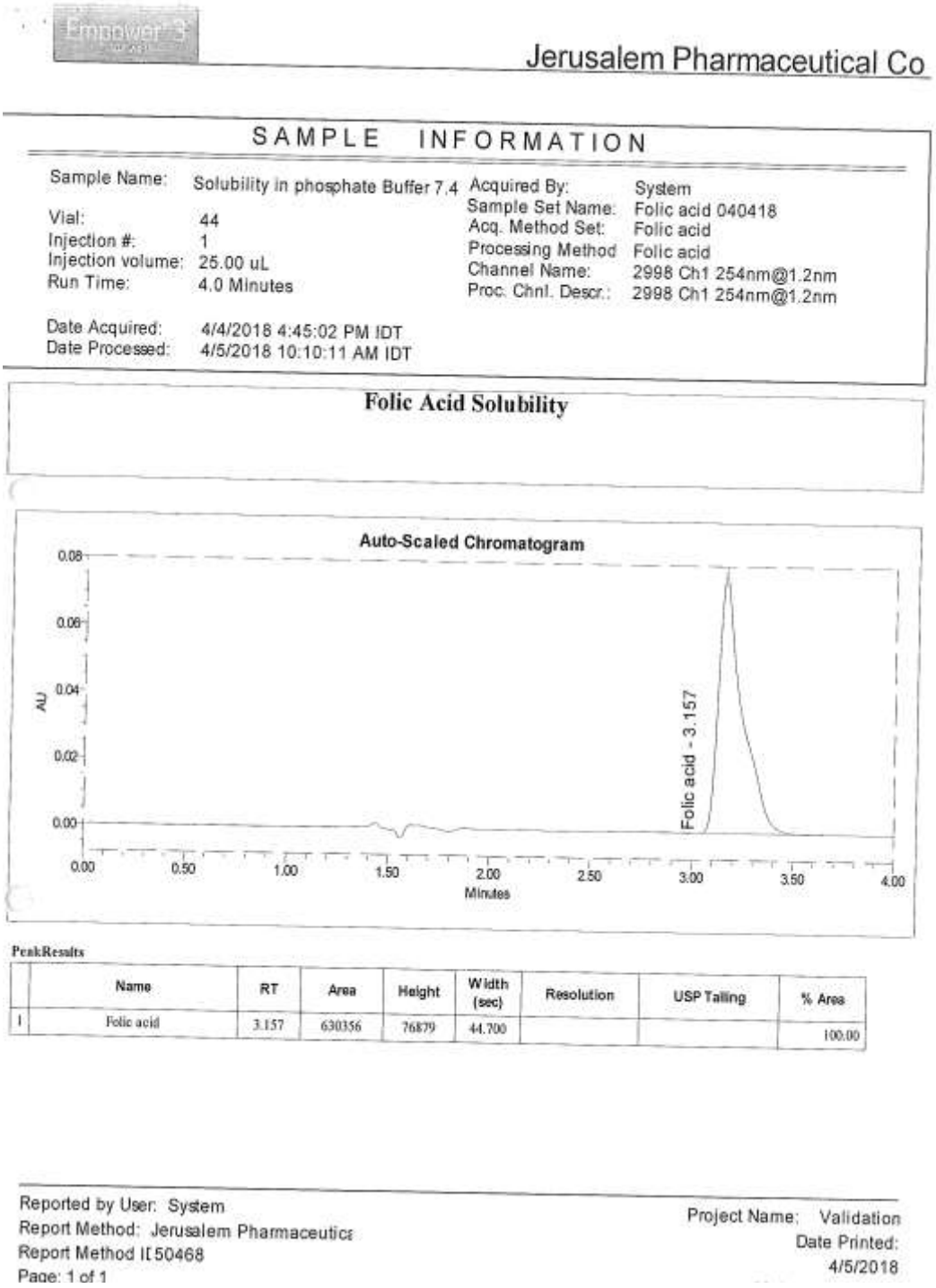

Figure 5.5: A chromatograph of Folic acid Solubility study in buffer pH 7.4 using Waters HPLC

Empower 3

Jerusalem Pharmaceutical

SAMPLE INFORMATION

Sample Name:	Solubility in 6.8 phosphate	Acquired By:	System
Sample Type:	Unknown	Sample Set Name	Folic acid 040418
Vial:	43	Acq. Method Set:	Folic acid
Injection #:	1	Processing Method	Folic acid
Injection Volume:	25.00 ul	Channel Name:	2998 Ch1 254nm@1.2nm
Run Time:	4.0 Minutes	Proc. Chnl. Descr.:	2998 Ch1 254nm@1.2nm
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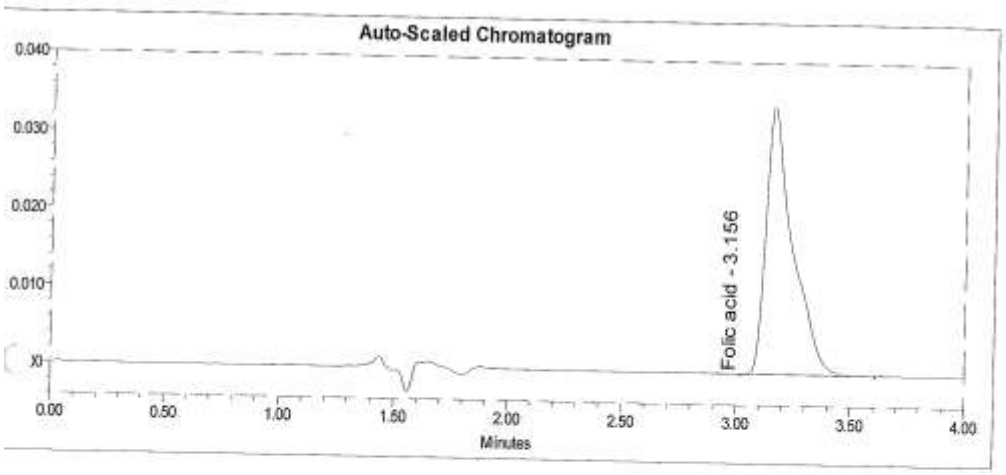
Folic Acid Solubility



Results

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Folic acid	3.156	281079	34435	35.600	

Reported by User: System
Report Method: Jerusalem Pharmaceutica
Report Method ID: 50465
Page: 1 of 1

Project Name: Validation
Date Printed: 4/5/2018

Figure 5.6: A chromatograph of Folic acid Solubility study in buffer pH 6.8 using Waters HPLC

Empower 3

Jerusalem Pharmaceutical

SAMPLE INFORMATION

Sample Name:	Solubility in Acetate Buffer 4.5	Acquired By:	System
Sample Type:	Unknown	Sample Set Name	Folic acid 040418
Vial:	42	Acq. Method Set:	Folic acid
Injection #:	1	Processing Method	Folic acid
Injection Volume:	25.00 ul	Channel Name:	2998 Ch1 254nm@1.2nm
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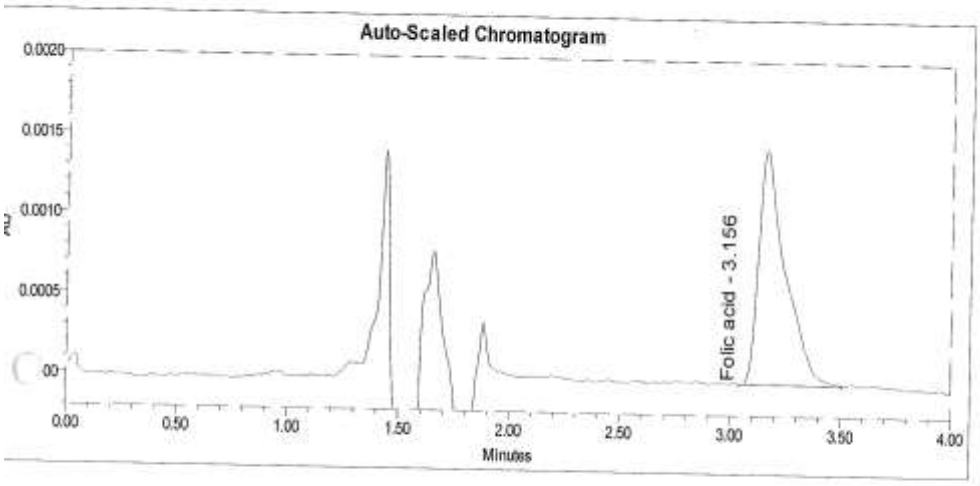
Folic Acid Solubility



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Reported by User: System
Report Method: Jerusalem Pharmaceutica
Report Method ID: 50466
Page: 1 of 1

Project Name: Validation
Date Printed: 4/5/2018

5.7: A chromatograph of Folic acid Solubility Figure study in buffer pH 4.5 using Waters HPLC

SAMPLE INFORMATION

Sample Name:	Solubility in 0.1%T.E.A	Acquired By:	System
Sample Type:	Unknown	Sample Set Name	Folic acid 040418
Vial:	41	Acq. Method Set:	Folic acid
Injection #:	1	Processing Method	Folic acid
Injection Volume:	25.00 ul	Channel Name:	2998 Ch1 254nm@1.2nm
Run Time:	4.0 Minutes	Proc. Chnl. Descr.:	2998 Ch1 254nm@1.2nm
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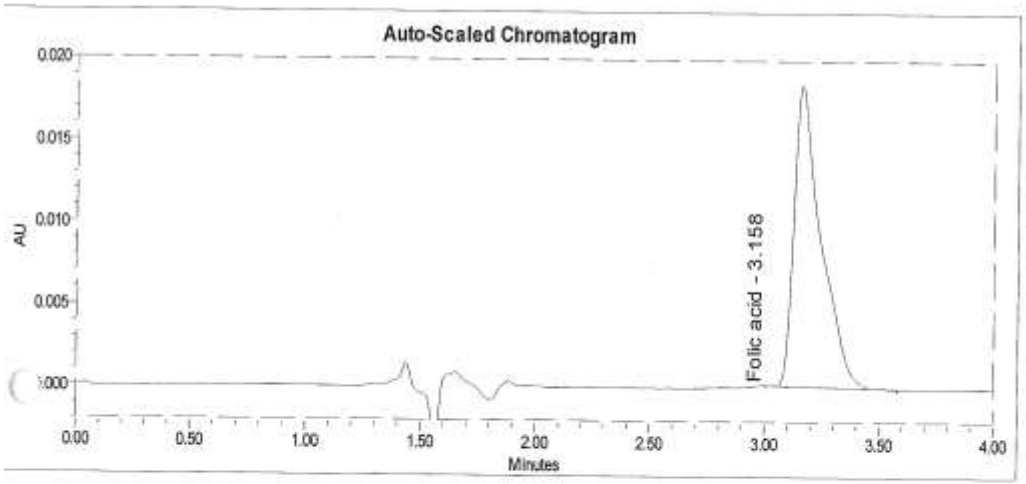
Folic Acid Solubility



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Reported by User: System
Report Method: Jerusalem Pharmaceutica
Report Method ID 50463

Project Name: Validation
Date Printed: 4/5/2018

Figure 5.8: A chromatograph of Folic acid Solubility study in 0.1% TEA using Waters HPLC

SAMPLE INFORMATION

Sample Name:	Solubility in 0.1%T.E.A	Acquired By:	System
Sample Type:	Unknown	Sample Set Name	Folic acid 040418
Vial:	41	Acq. Method Set:	Folic acid
Injection #:	1	Processing Method	Folic acid
Injection Volume:	25.00 ul	Channel Name:	2998 Ch1 254nm@1.2nm
Run Time:	4.0 Minutes	Proc. Chnl. Descr.:	2998 Ch1 254nm@1.2nm
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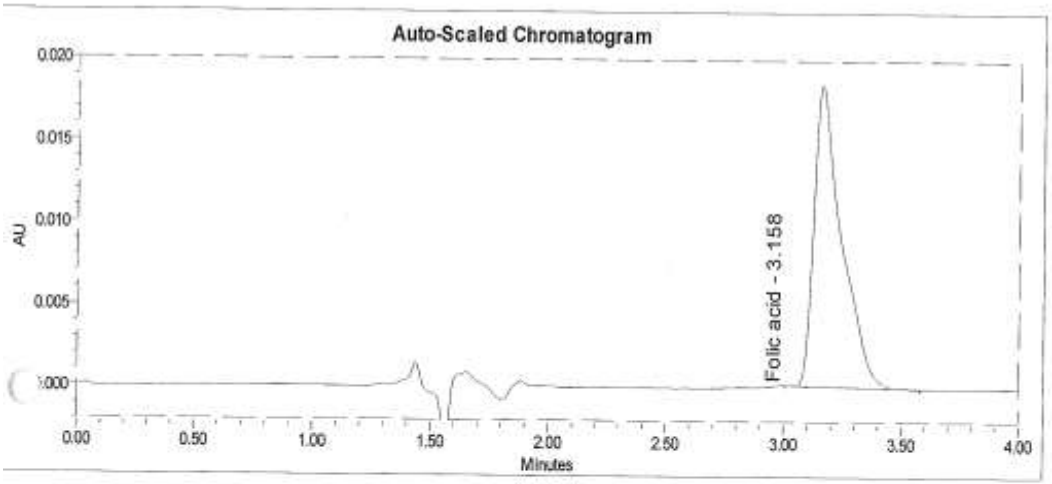
Folic Acid Solubility



kResults

Name	RT	Area	Height	Width (sec)	Resolution
Folic acid	3.158	150156	18431	34.400	

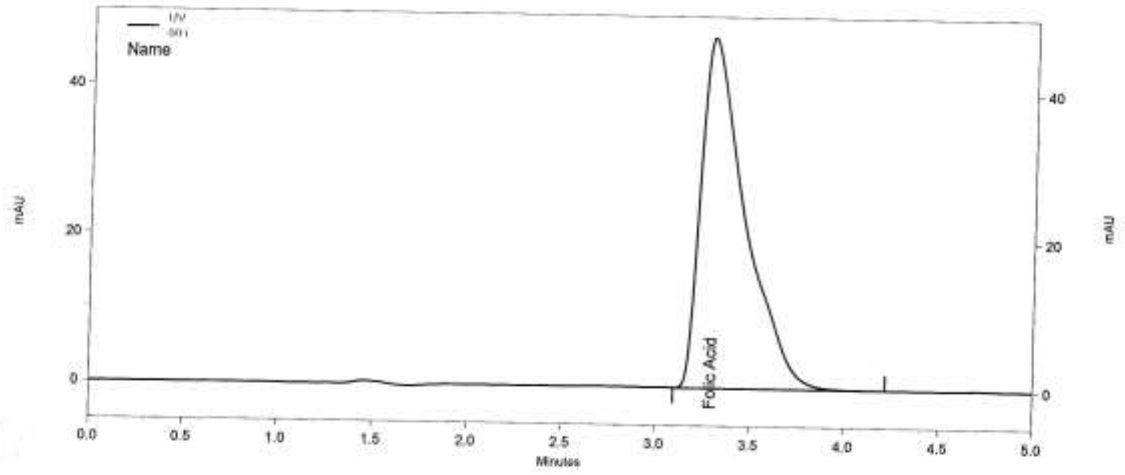
Reported by User: System
Report Method: Jerusalem Pharmaceutica
Report Method ID: 50463

Project Name: Validation
Date Printed: 4/5/2018

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\040418\001-Rep1.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *04-Apr-18 10:16:10*
Analysis Time: *04-Apr-18 11:19:17*
Injection Volume: *25*
Vial Number: *101*
Print Time: *07-Apr-18 11:47:03*
Data Description: *STD ASSAY*

Folic acid Gel (F1)
ASSAY



UV Results

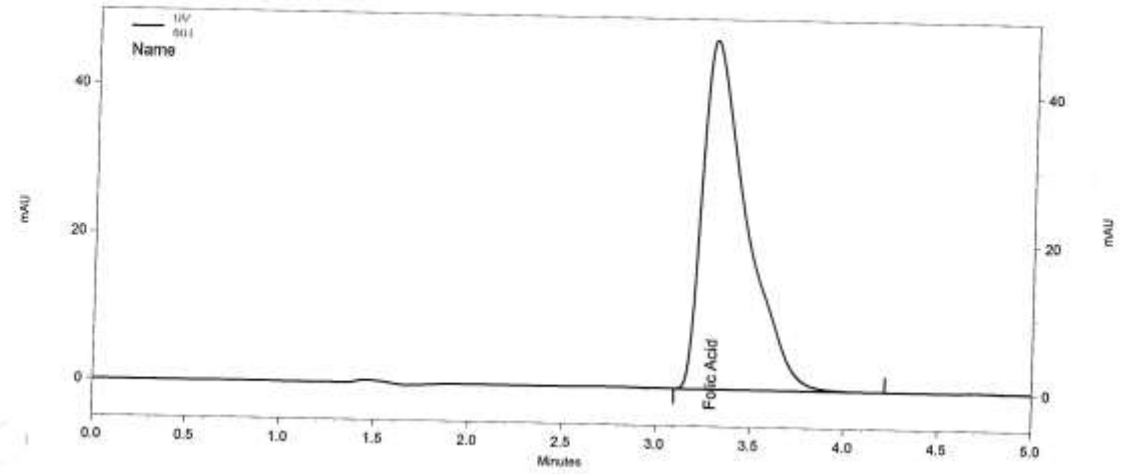
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*3.29*	*3078882*	*100.00*	*188361*
Totals			*3078882*	*100.00*	*188361*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\040418\001-Rep2.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *04-Apr-18 10:22:27*
Analysis Time: *04-Apr-18 10:27:29*
Injection Volume: *25*
Vial Number: *101*
Print Time: *07-Apr-18 11:47:24*
Data Description: *STD ASSAY*

Folic acid Gel (F1)
ASSAY



UV Results

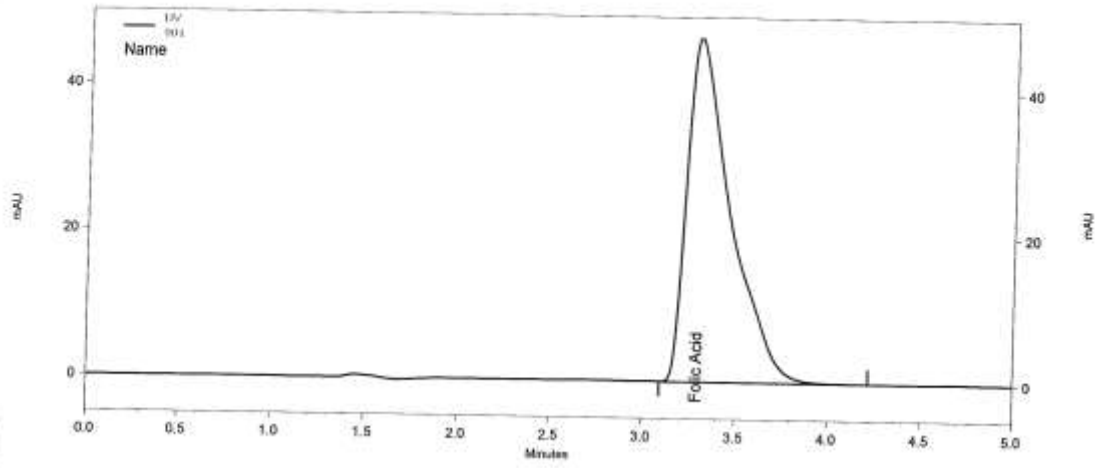
Pk #	Name	Retention Time	Area	Area %	Height
1	*Folic Acid*	*3.27*	*3086382*	*100.00*	*176148*
Totals			*3086382*	*100.00*	*176148*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\040418\001-Rep3.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *04-Apr-18 10:28:39*
Analysis Time: *04-Apr-18 10:33:51*
Injection Volume: *25*
Vial Number: *101*
Print Time: *07-Apr-18 11:47:36*
Data Description: *STD ASSAY*

Folic acid Gel (F1)
ASSAY



UV Results

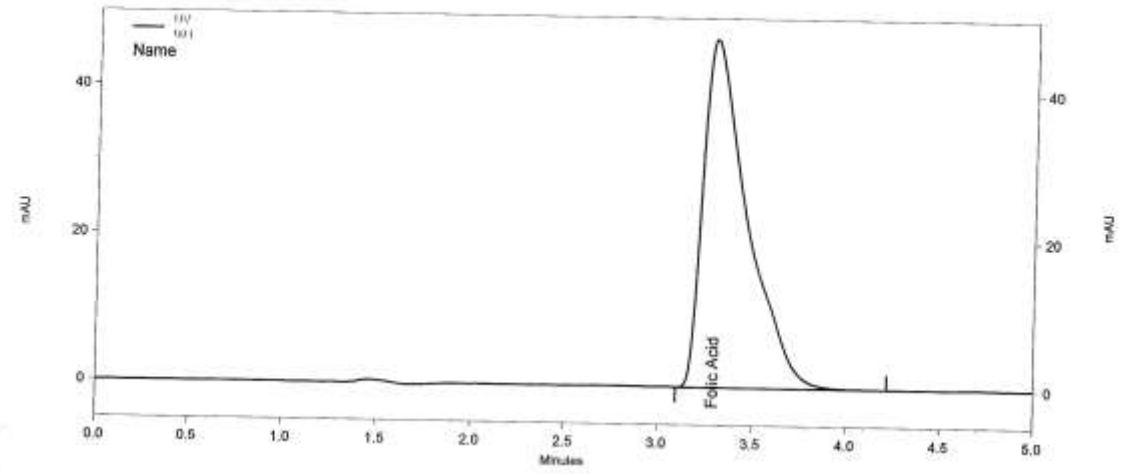
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*3.27*	*3088052*	*100.00*	*168566*
Totals			*3088052*	*100.00*	*168566*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\040418\001-Rep4.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *04-Apr-18 10:35:02*
Analysis Time: *04-Apr-18 10:40:15*
Injection Volume: *25*
Vial Number: *101*
Print Time: *07-Apr-18 11:47:41*
Data Description: *STD ASSAY*

Folic acid Gel (F1)
ASSAY



UV Results

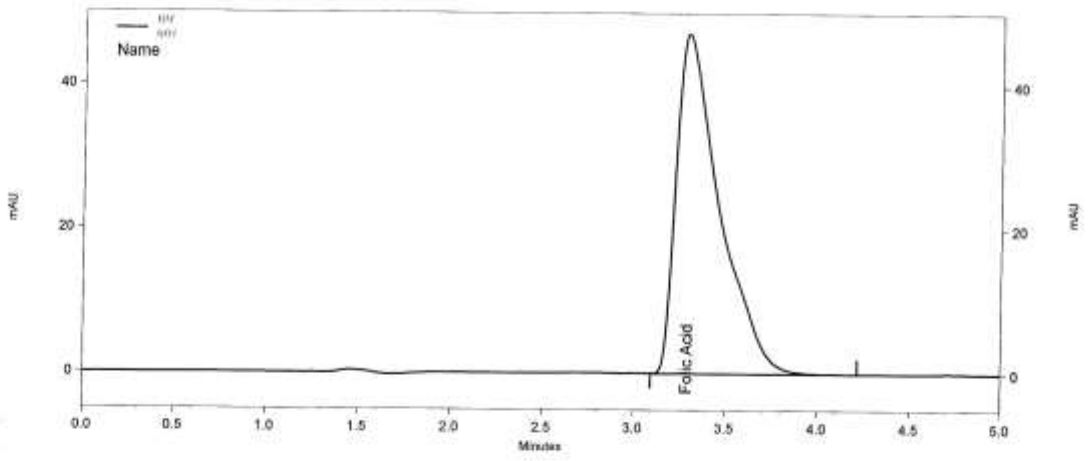
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*3.27*	*3086153*	*100.00*	*163003*
Totals			*3086153*	*100.00*	*163003*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\040418\001-Rep5.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *04-Apr-18 10:41:19*
Analysis Time: *04-Apr-18 10:46:30*
Injection Volume: *25*
Vial Number: *101*
Print Time: *07-Apr-18 11:47:47*
Data Description: *STD ASSAY*

Folic acid Gel (F1)
ASSAY



UV Results

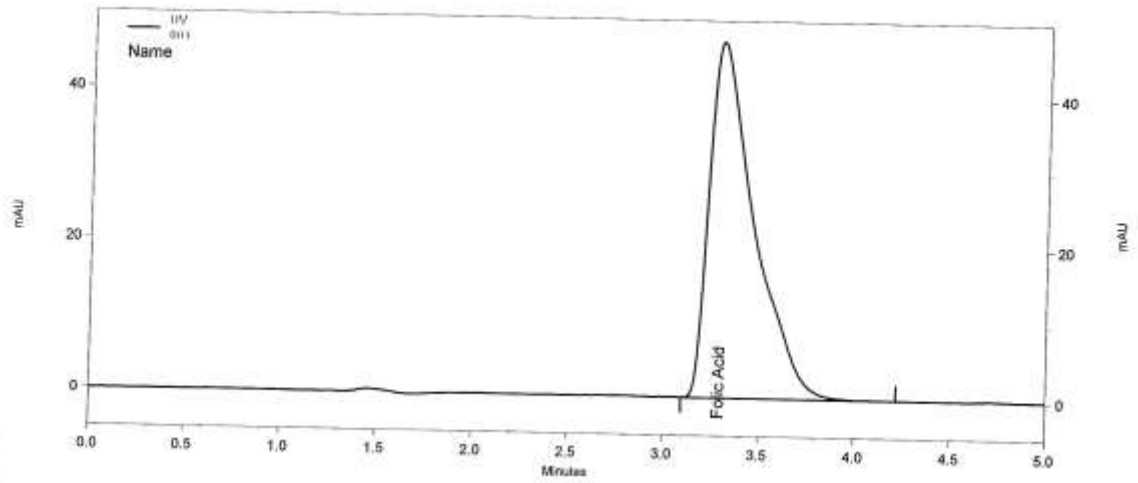
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*3.26*	*3096004*	*100.00*	*160771*
Totals			*3096004*	*100.00*	*160771*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\040418\003.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *003*
Run Time: *04-Apr-18 11:00:12*
Analysis Time: *04-Apr-18 11:18:39*
Injection Volume: *25*
Vial Number: *101*
Print Time: *07-Apr-18 11:48:03*
Data Description: *STD ASSAY*

Folic acid Gel (F1)
ASSAY



UV Results

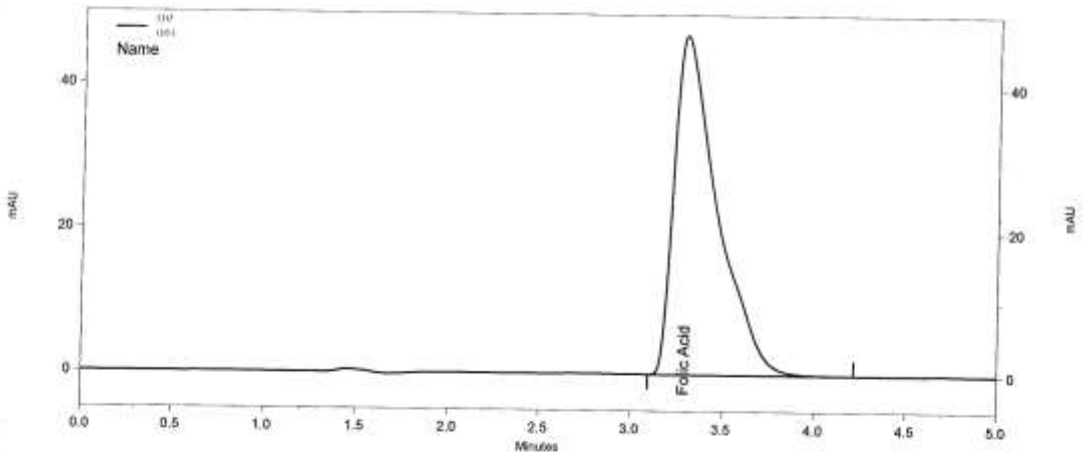
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*3.26*	*3091531*	*100.00*	*151455*
Totals			*3091531*	*100.00*	*151455*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\040418\002-Rep1.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *002*
Run Time: *04-Apr-18 10:47:34*
Analysis Time: *04-Apr-18 10:52:45*
Injection Volume: *25*
Vial Number: *102*
Print Time: *07-Apr-18 11:48:24*
Data Description: *ASSAY Gel F1*

Folic acid Gel (F1)
ASSAY



UV Results

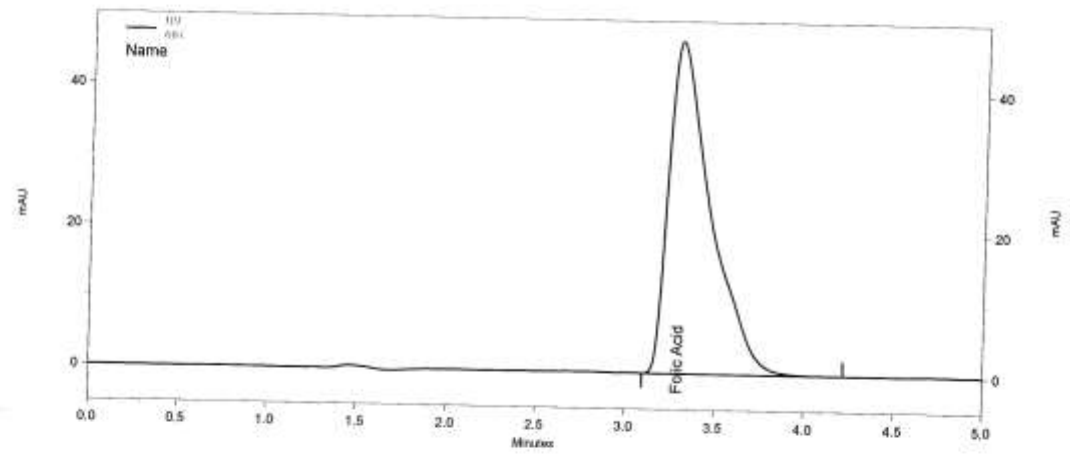
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*3.26*	*3141606*	*100.00*	*159583*
Totals			*3141606*	*100.00*	*159583*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\040418\002-Rep2.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *002*
Run Time: *04-Apr-18 10:53:56*
Analysis Time: *04-Apr-18 11:23:25*
Injection Volume: *25*
Vial Number: *102*
Print Time: *07-Apr-18 11:48:39*
Data Description: *ASSAY Gel F1*

Folic acid Gel (F1)
ASSAY



UV Results

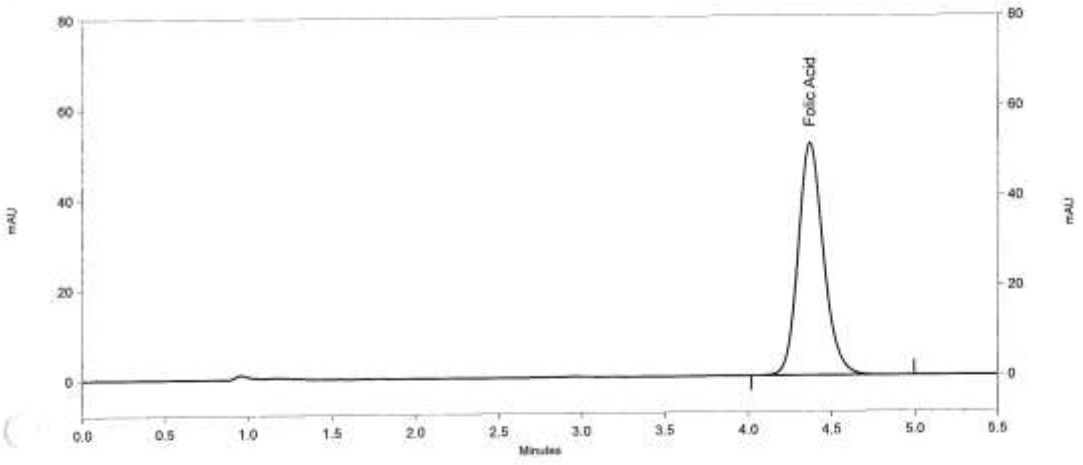
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*3.26*	*3155239*	*100.00*	*154659*
Totals			*3155239*	*100.00*	*154659*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\180418\001-Rep1.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *18-Apr-18 14:43:31*
Analysis Time: *18-Apr-18 15:20:15*
Injection Volume: *25*
Vial Number: *101*
Print Time: *18-Apr-18 15:20:33*
Data Description: *Folic Acid Std"0.02mg/ml"*

Folic acid Gel (F1)
Stability"After 14 Days"



UV Results

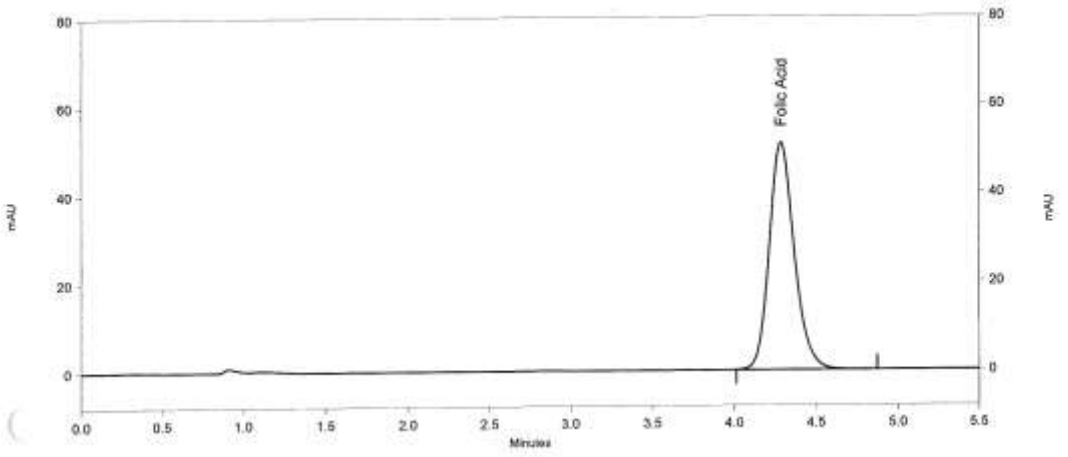
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*4.37*	*2177952*	*100.00*	*206644*
Totals			*2177952*	*100.00*	*206644*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\180418\001-Rep2.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *18-Apr-18 14:50:18*
Analysis Time: *18-Apr-18 15:20:39*
Injection Volume: *25*
Vial Number: *101*
Print Time: *18-Apr-18 15:20:41*
Data Description: *Folic Acid Std"0.02mg/ml"*

Folic acid Gel (F1)
Stability"After 14 Days"



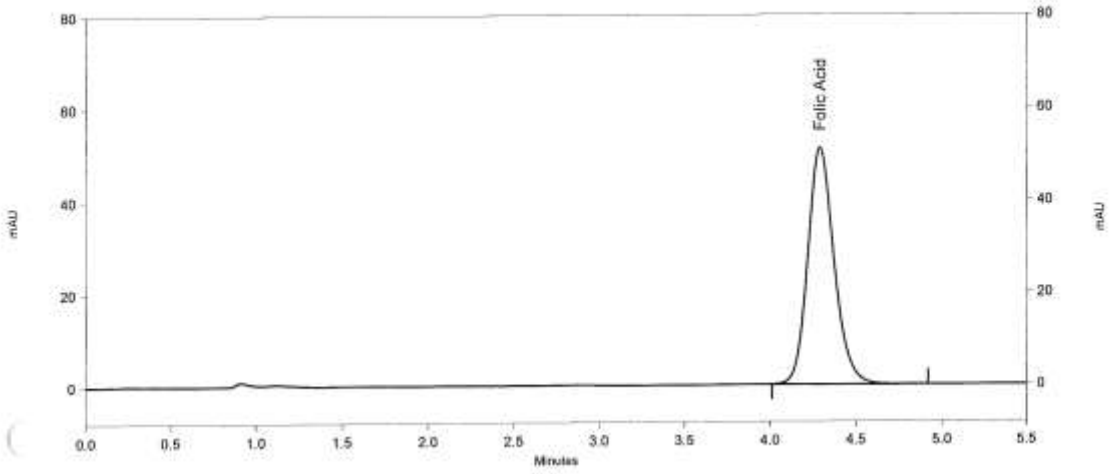
UV Results Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*4.28*	*2171440*	*100.00*	*204994*
Totals			*2171440*	*100.00*	*204994*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\180418\001-Rep3.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *18-Apr-18 14:57:06*
Analysis Time: *18-Apr-18 15:20:46*
Injection Volume: *25*
Vial Number: *101*
Print Time: *18-Apr-18 15:20:47*
Data Description: *Folic Acid Std"0.02mg/ml"*

Folic acid Gel (F1)
Stability"After 14 Days"



UV Results

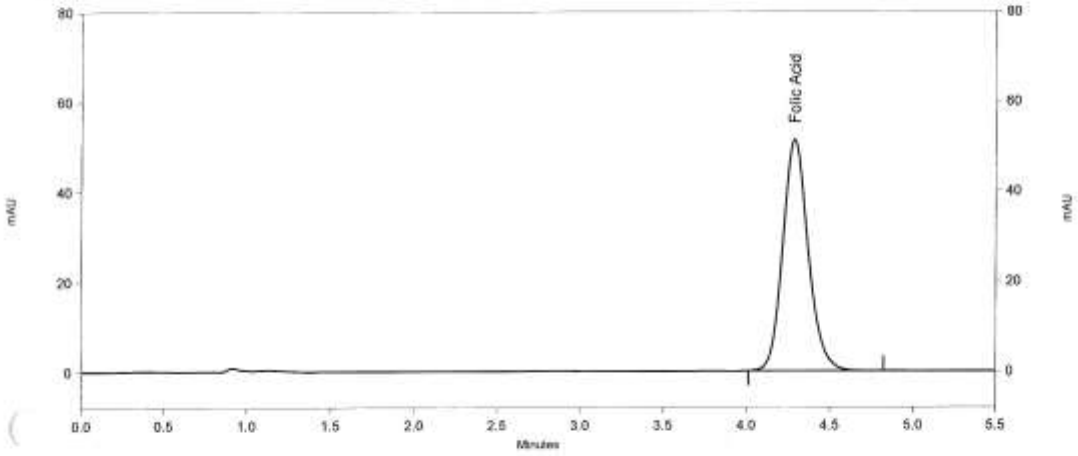
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*4.29*	*2172442*	*100.00*	*204752*
Totals			*2172442*	*100.00*	*204752*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\180418\001-Rep4.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *18-Apr-18 15:03:50*
Analysis Time: *18-Apr-18 15:20:54*
Injection Volume: *25*
Vial Number: *101*
Print Time: *18-Apr-18 15:20:55*
Data Description: *Folic Acid Std"0.02mg/ml"*

Folic acid Gel (F1)
Stability"After 14 Days"



UV Results

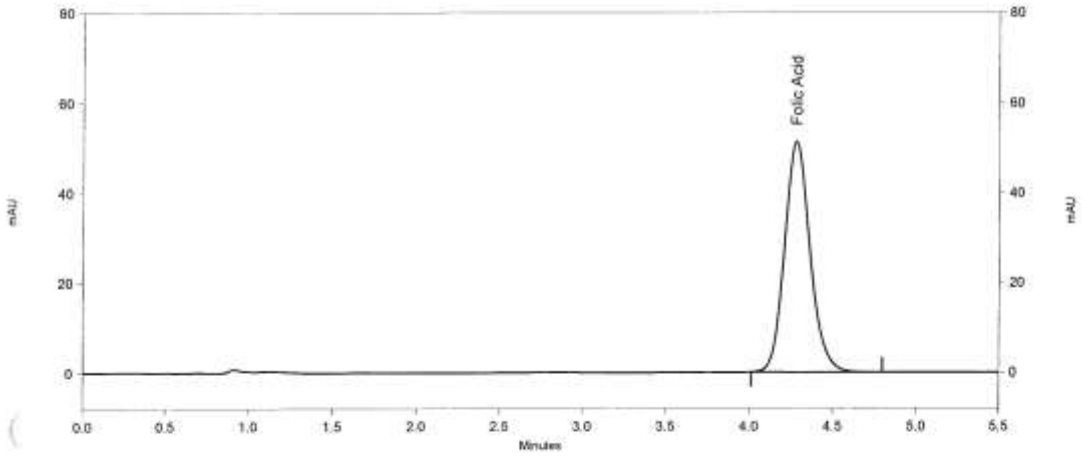
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*4.29*	*2174794*	*100.00*	*205103*
Totals			***2174794***	***100.00***	***205103***

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\180418\001-Rep5.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *18-Apr-18 15:10:38*
Analysis Time: *18-Apr-18 15:21:02*
Injection Volume: *25*
Vial Number: *101*
Print Time: *18-Apr-18 15:21:04*
Data Description: *Folic Acid Std"0.02mg/ml"*

Folic acid Gel (F1)
Stability"After 14 Days"



UV Results

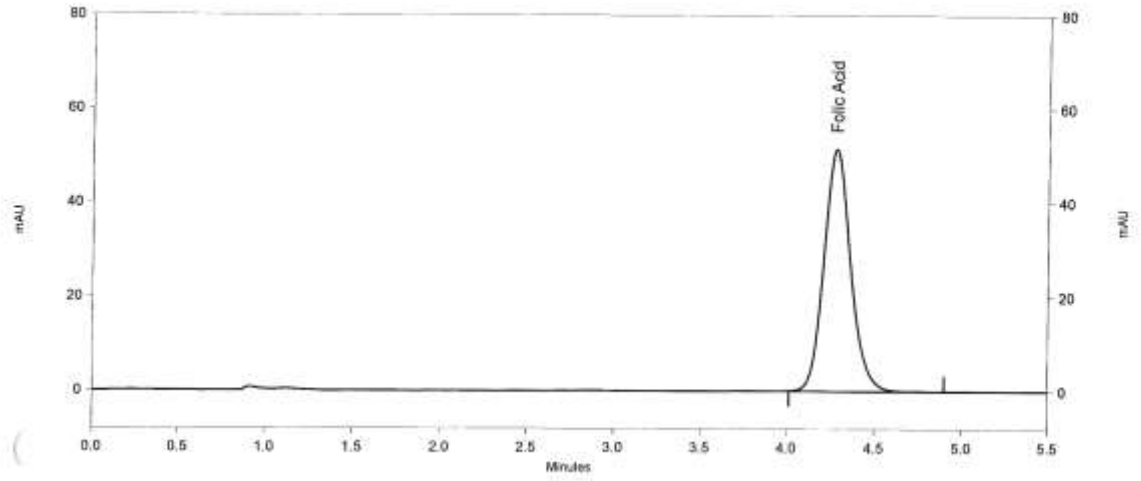
Pk #	***Name***	***Retention Time***	***Area***	***Area %***	***Height***
1	*Folic Acid*	*4.28*	*2173547*	*100.00*	*205268*
Totals			***2173547***	***100.00***	***205268***

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\180418\001-Rep6.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *18-Apr-18 15:17:29*
Analysis Time: *18-Apr-18 15:23:14*
Injection Volume: *25*
Vial Number: *101*
Print Time: *18-Apr-18 15:23:15*
Data Description: *Folic Acid Std"0.02mg/ml"*

Folic acid Gel (F1)
Stability"After 14 Days"



UV Results

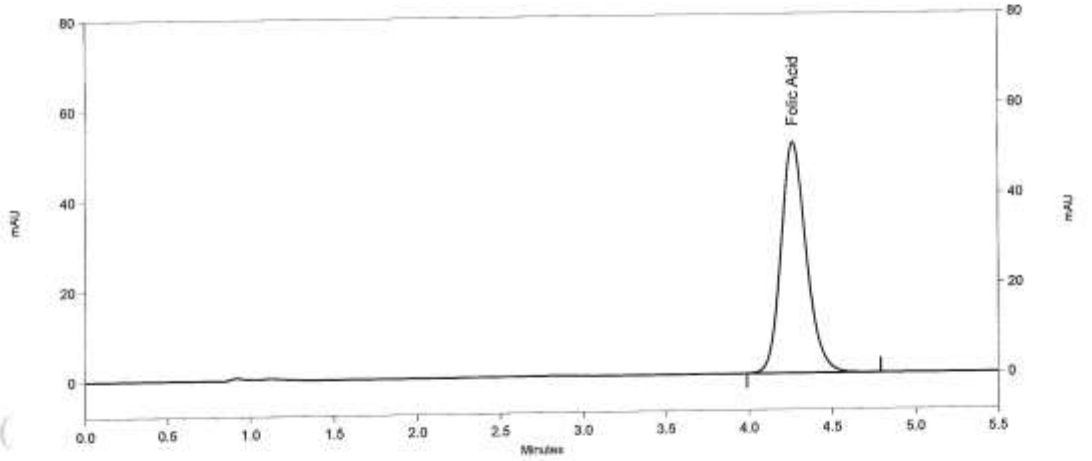
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*4.28*	*2171256*	*100.00*	*205293*
Totals			*2171256*	*100.00*	*205293*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\180418\001.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *18-Apr-18 15:45:50*
Analysis Time: *18-Apr-18 15:50:53*
Injection Volume: *25*
Vial Number: *101*
Print Time: *18-Apr-18 15:51:24*
Data Description: *Folic Acid Std"0.02mg/ml"*

Folic acid Gel (F1)
Stability"After 14 Days"



UV Results

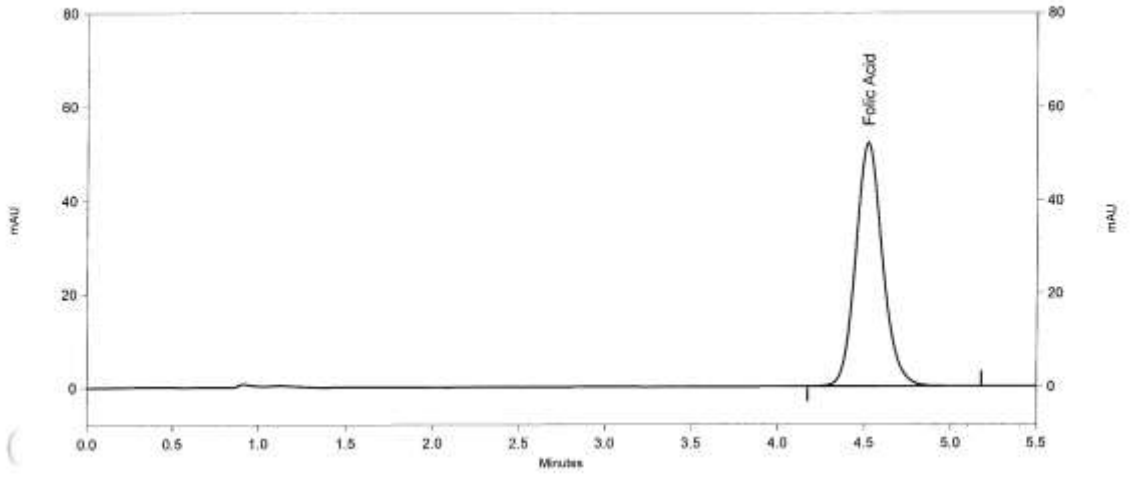
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*4.26*	*2174138*	*100.00*	*205640*
Totals			*2174138*	*100.00*	*205640*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\180418\002.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *002*
Run Time: *18-Apr-18 15:24:12*
Analysis Time: *18-Apr-18 15:32:32*
Injection Volume: *25*
Vial Number: *102*
Print Time: *18-Apr-18 15:32:50*
Data Description: *ASSAY F1 Gel At 25C+60%RH*

Folic acid Gel (F1)
Stability"After 14 Days"



UV Results

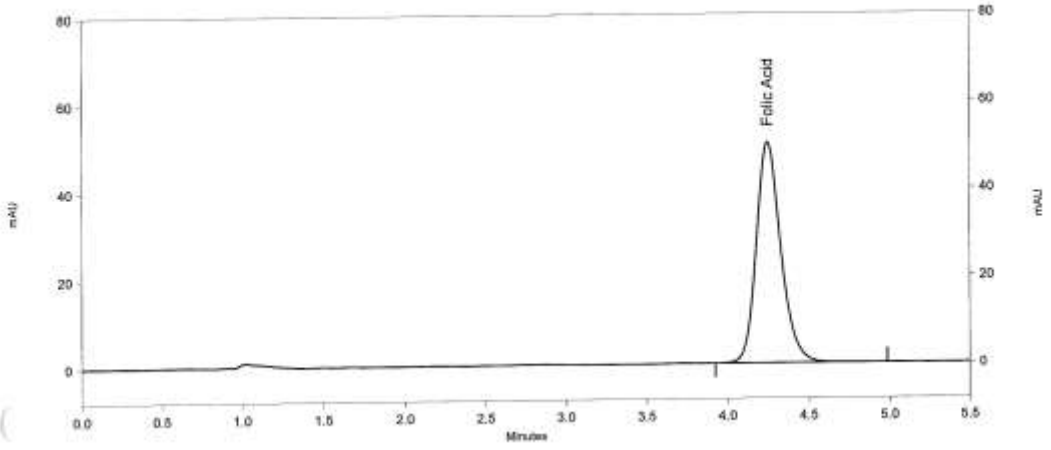
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*4.52*	*2258960*	*100.00*	*208576*
Totals			*2258960*	*100.00*	*208576*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\180418\003.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *003*
Run Time: *18-Apr-18 15:30:55*
Analysis Time: *18-Apr-18 15:36:58*
Injection Volume: *25*
Vial Number: *103*
Print Time: *18-Apr-18 15:37:02*
Data Description: *ASSAY F1 Gel At 30C+65%RH*

Folic acid Gel (F1)
Stability"After 14 Days"



UV Results

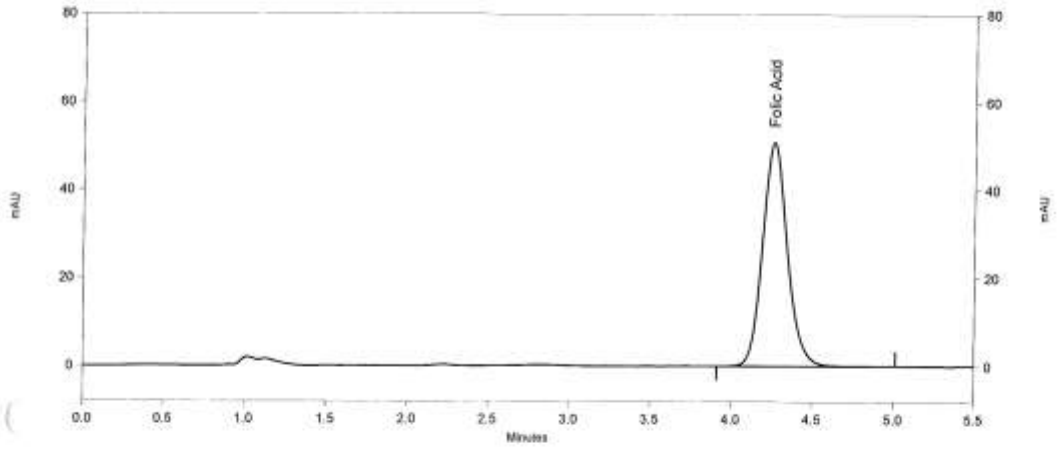
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*4.25*	*2120042*	*100.00*	*201614*
Totals			*2120042*	*100.00*	*201614*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\180418\004.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *004*
Run Time: *18-Apr-18 15:37:28*
Analysis Time: *18-Apr-18 15:43:36*
Injection Volume: *25*
Vial Number: *104*
Print Time: *18-Apr-18 15:43:39*
Data Description: *ASSAY F1 Gel At 40C+75%RH*

Folic acid Gel (F1)
Stability"After 14 Days"



UV Results

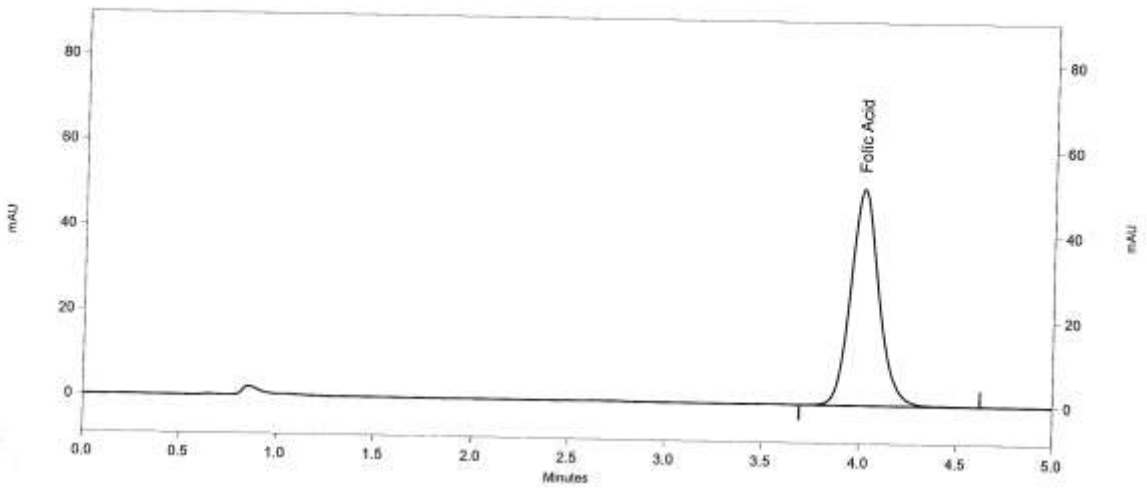
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*4.26*	*2154924*	*100.00*	*202998*
Totals			***2154924***	***100.00***	***202998***

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\160518\001.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *16-May-18 12:55:27*
Analysis Time: *16-May-18 13:05:32*
Injection Volume: *25*
Vial Number: *101*
Print Time: *16-May-18 14:18:57*
Data Description: *Folic Acid Std"0.02mg/ml"*

Folic acid Gel (F1)
Stability"After 42 Days"



UV Results

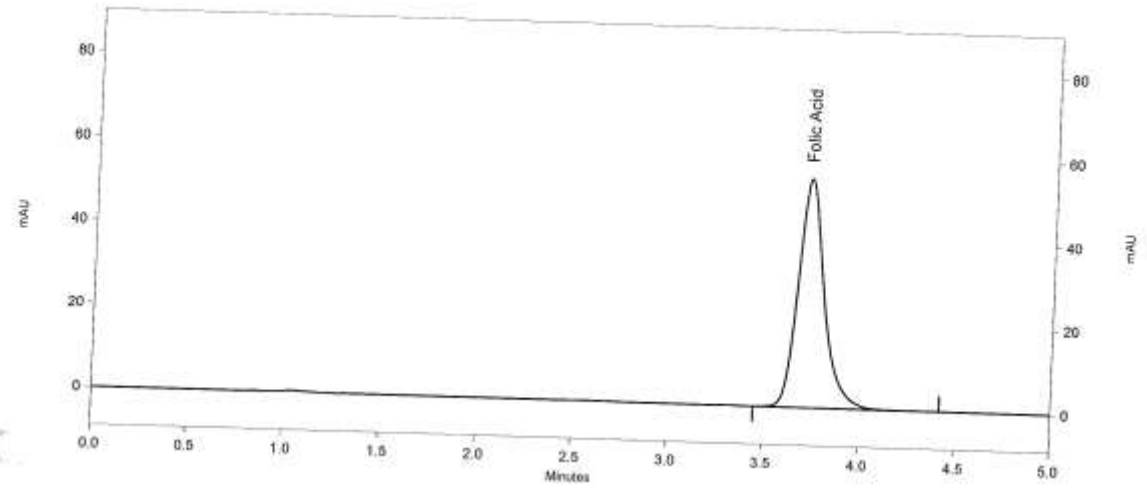
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*4.01*	*2054304*	*100.00*	*203047*
Totals			*2054304*	*100.00*	*203047*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\160518\001-Rep1.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *16-May-18 12:24:06*
Analysis Time: *16-May-18 13:06:03*
Injection Volume: *25*
Vial Number: *101*
Print Time: *16-May-18 14:19:17*
Data Description: *Folic Acid Std"0.02mg/ml"*

Folic acid Gel (F1)
Stability"After 42 Days"



UV Results

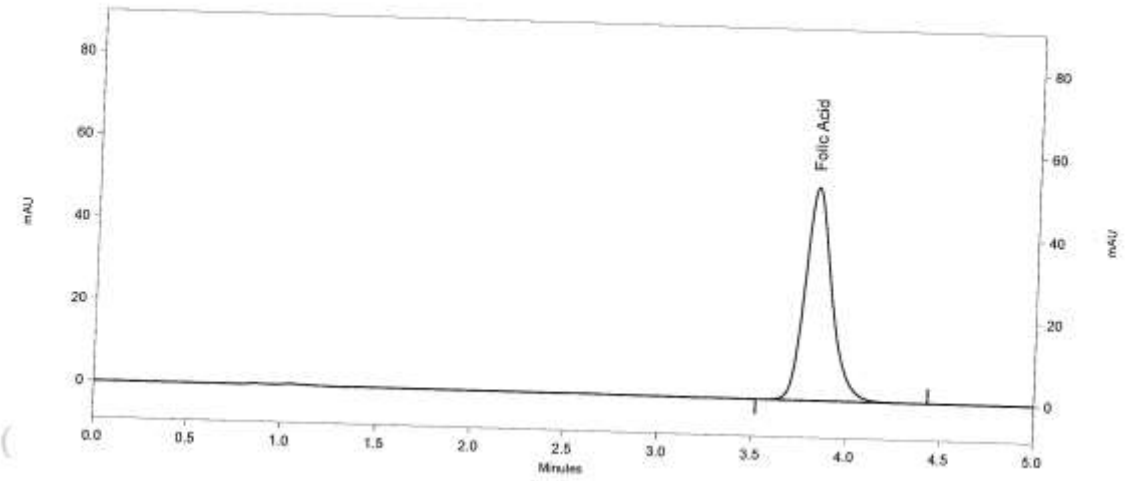
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*3.72*	*2056356*	*100.00*	*218356*
Totals			*2056356*	*100.00*	*218356*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\160518\001-Rep2.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *16-May-18 12:30:17*
Analysis Time: *16-May-18 13:06:14*
Injection Volume: *25*
Vial Number: *101*
Print Time: *16-May-18 14:19:28*
Data Description: *Folic Acid Std"0.02mg/ml"*

Folic acid Gel (F1)
Stability"After 42 Days"



UV Results

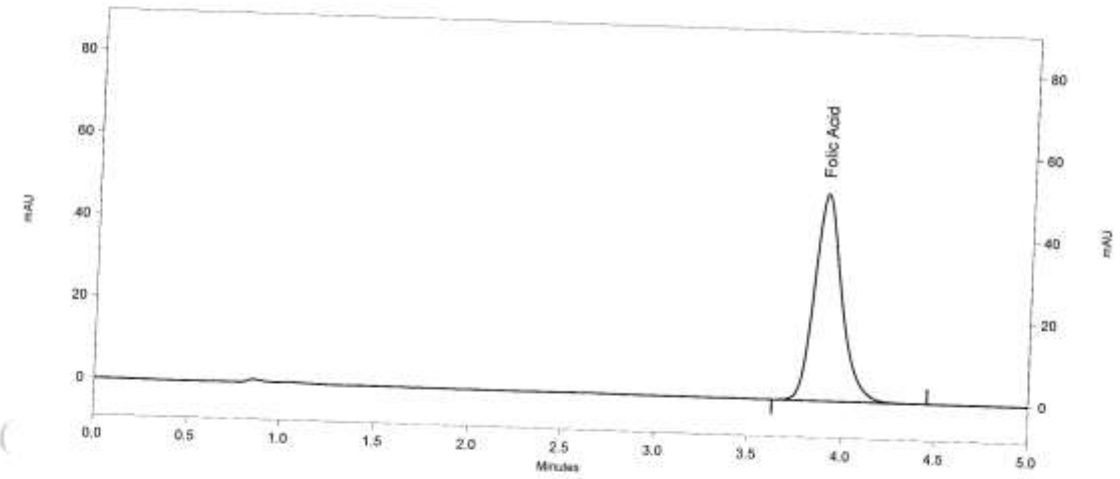
Pk #	Name	Retention Time	Area	Area %	Height
1	*Folic Acid*	*3.83*	*2049479*	*100.00*	*206963*
Totals			*2049479*	*100.00*	*206963*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\160518\001-Rep3.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *16-May-18 12:36:32*
Analysis Time: *16-May-18 13:06:24*
Injection Volume: *25*
Vial Number: *101*
Print Time: *16-May-18 14:19:36*
Data Description: *Folic Acid Std"0.02mg/ml"*

Folic acid Gel (F1)
Stability"After 42 Days"



UV Results

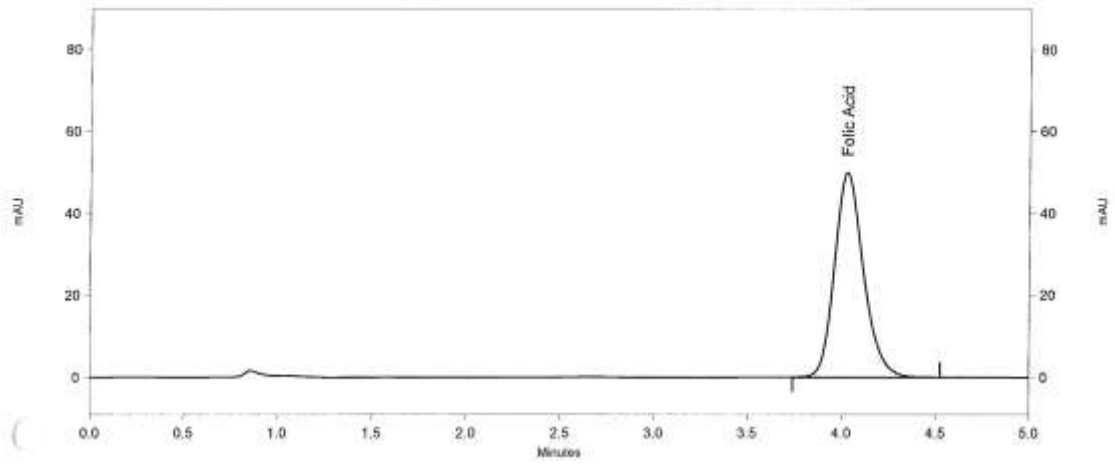
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*3.90*	*2045726*	*100.00*	*201303*
Totals			*2045726*	*100.00*	*201303*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\160518\001-Rep4.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *16-May-18 12:42:47*
Analysis Time: *16-May-18 14:22:08*
Injection Volume: *25*
Vial Number: *101*
Print Time: *16-May-18 14:22:16*
Data Description: *Folic Acid Std"0.02mg/ml"*

Folic acid Gel (F1)
Stability"After 42 Days"



UV Results

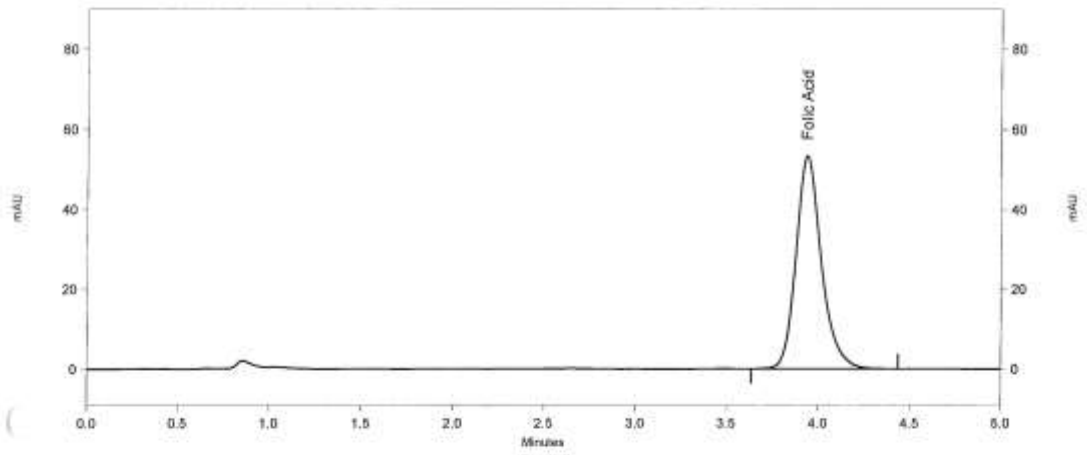
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*4.03*	*2043880*	*100.00*	*196570*
Totals			*2043880*	*100.00*	*196570*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\160518\001-Rep5.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *001*
Run Time: *16-May-18 12:49:06*
Analysis Time: *16-May-18 13:06:48*
Injection Volume: *25*
Vial Number: *101*
Print Time: *16-May-18 14:19:48*
Data Description: *Folic Acid Std"0.02mg/ml"*

Folic acid Gel (F1)
Stability"After 42 Days"



UV Results

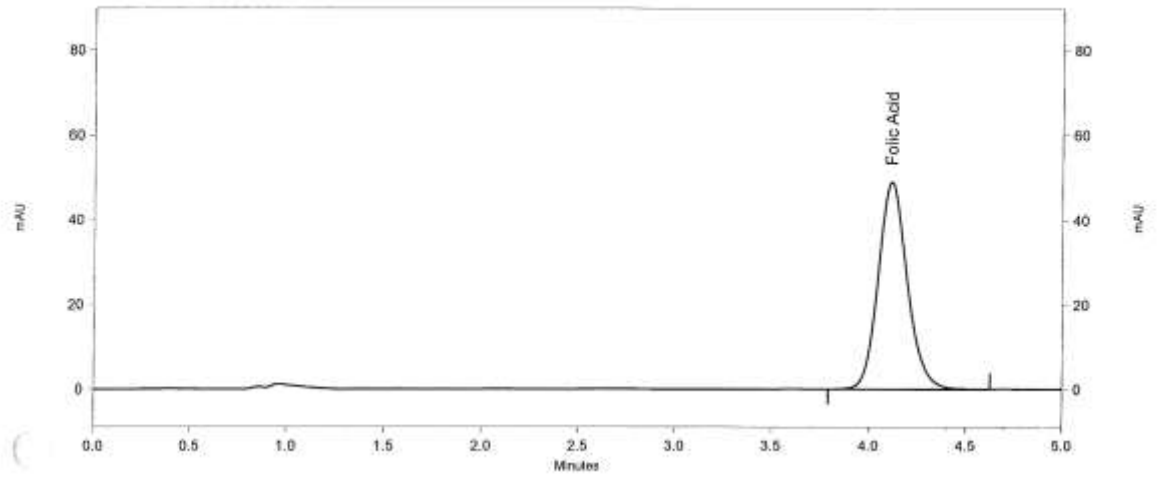
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*3.94*	*2049015*	*100.00*	*213247*
Totals			*2049015*	*100.00*	*213247*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\160518\04.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *004*
Run Time: *16-May-18 14:02:26*
Analysis Time: *16-May-18 14:07:55*
Injection Volume: *25*
Vial Number: *104*
Print Time: *16-May-18 14:23:38*
Data Description: *ASSAY F1 Gel At 25C+60%RH*

Folic acid Gel (F1)
Stability"After 42 Days"



UV Results

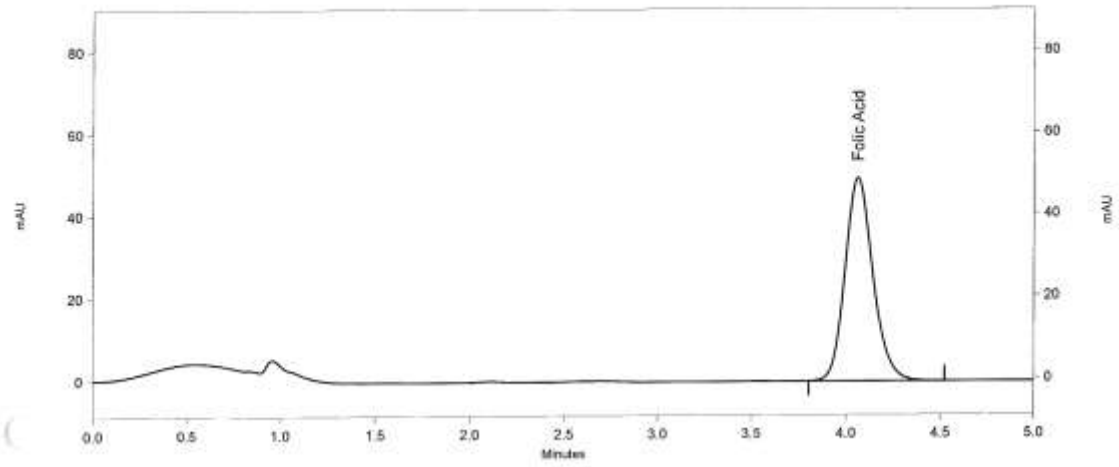
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*4.11*	*2047811*	*100.00*	*195624*
Totals			*2047811*	*100.00*	*195624*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\160518\02.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *002*
Run Time: *16-May-18 13:49:49*
Analysis Time: *16-May-18 13:55:00*
Injection Volume: *25*
Vial Number: *103*
Print Time: *16-May-18 14:33:16*
Data Description: *ASSAY F1 Gel At 30C+65%RH*

Folic acid Gel (F1)
Stability"After 42 Days"



UV Results

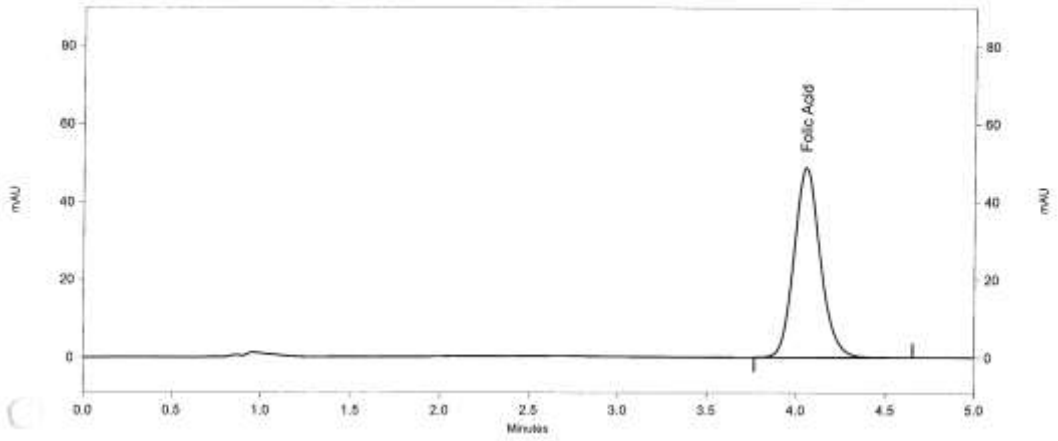
Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*4.06*	*2025530*	*100.00*	*197605*
Totals			*2025530*	*100.00*	*197605*

Analyzed By :

Jerusalem Pharmaceuticals Co. Ltd.
R&D Department

File Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Data\Folic Acid\160518\004.dat*
Method Name: *C:\EZChrom Elite\Enterprise\Projects\Stability\Method\Folic Acid.met*
Sample ID: *004*
Run Time: *16-May-18 14:09:47*
Analysis Time: *16-May-18 14:28:07*
Injection Volume: *25*
Vial Number: *102*
Print Time: *16-May-18 14:28:26*
Data Description: *ASSAY F1 Gel At 40C+75%RH*

Folic acid Gel (F1)
Stability"After 42 Days"



UV Results

Pk #	*Name*	*Retention Time*	*Area*	*Area %*	*Height*
1	*Folic Acid*	*4.05*	*1995580*	*100.00*	*194722*
Totals			*1995580*	*100.00*	*194722*

Analyzed By :

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تحضير حامض الفوليك على شكل جل موضعي و التحقق من تأثير محسنات النفاذية على درجة نفاذيته

إعداد: أسامة الفار

المشرف: د. مؤمل قرط

المشرف المشارك: د. نعمان مالكية

ملخص

بعتير حامض الفوليك من أجد مكونات مجموعة فيتامينات- ب ,ويؤدي دورًا في إنتاج ونضج خلايا الدم الحمراء بشكل طبيعي. وهو متوفر في الأسواق على شكل صيدلاني أقراص لوحده أو مع مركبات الحديد وفيتامينات- ب الأخرى. كما أن حمض الفوليك (FA) له خصائص تجديد الجلد عن طريق إصلاح الحمض النووي في الجلد المتضرر من الأشعة فوق البنفسجية عن طريق تحسين مرونة البشرة وترطيبها ، وتقليل خشونة الجلد عبر تقليل فقدان المياه عبر الجلد و دون أي تغيير كبير في إفراز الزهم.

تعد المستحضرات الموضعية لحامض الفوليك مستحضرات واعدة بسبب سهولة إستعمالها ومقبوليتها من قبل المرضى ، ولكنها ليست بالقدر الكافي من حيث الفعالة لأنها لا تخترق الجلد بشكل جيد .

الهدف من هذه الأطروحة هو دراسة تأثير بعض معززات الاختراق على نفاذية أو إختراق حامض الفوليك مطور على شكل هلام أو جل، كنموذج لأحد الأشكال الموضعية شبه الصلبة

في المرحلة الأولى تم تحديد معلمات الإنتشار لتجربة هلام فوليـــك تركيـــز (0.1٪ وزن / وزن) واستخدامها كمرجع لقياس آثار مختلف معززات الاختراق على نفاذيته.

في المرحلة الثانية من البحث تم استخدام عينة سليمة من جلد الخنزير لفحص نفاذية أفضـــل نظام منتقى من الجل و محسنات النفاذية.

في المرحلة الأولى ، يتكون الغشاء من غشاء قشرة البيض الطبيعي المنقوع في الآيزوبروبيل ميريستات, حيث يتم تعبئة حجرة الإستقبال بمحلول منظم ذو رقم هيدروجيني له 7.4, بينما حجرة المرسل تحتوي على 5 غرام من الجل.

في المرحلة الثانية ، تم استخدام جلد خنزير تم الحصول عليه من أنثى جلد خنزير عمرها 9 أسابيع, وذلك لفصل حجرة الإرسال عن حجرة الاستقبال .تم أخذ عينات حجم 1 مل مـــن حجرة الإستقبال بعد كل ساعة لمدة سبع ساعات لكل عينة بحثية., ثم تلاها تحديـــد الكميـــة النافذة من حامض الفوليك في كل عينة وذلك من خلال تحليلها باستخدام جهاز HPLC على λ= 254 نانوميتر.

لقد تم تحديد ذائبية حامض الفوليك في محاليل أسيتات منظم ذو درجة حموضة 4.5 و في محاليل فوسفات على عدة درجات من الحموضة, وتبين أن الذائبية العظمى كانت في محلول فوسفات منظم ذو درجة حموضة 7.4، وبلغت (0.680 غم\ 100مل) .

تم اختبار توافق حمض الفوليك مع الســـواغات المختلفـــة (كحـــول البنزيـــل، كـــاربوبول، آيزوبروبيل ميريستات، توين 80، PSE-15، صوديوم لوريل سلفات، كحول أيزوبروبيل ، و(TEA ثلاثي إيثانول أمين لمدة ثلاثة أيام على درجة حرارة الغرفة و 40 درجة مئويـــة.

حيث تبين أن حامض الفوليك متوافق مع جميع السواغات إلا مـــع كـــاربوبول الـــذي أدى ترسيب المادة .

معززات الاختراق (PE) قيد الدراسة هي كحول البنزيل ، تـــوين 20 ، صـــوديوم لوريـــل سلفات ، PSE-15 ، آيزوبروبيل ميريستات وIPA كحول أيزوبروبيل، حيث تم إضـــافتها بتركيزات مختلفة إلى جل حامض فوليك تركيز 0.1٪.

تم الدراسة من خلية فرانز وتم تحديد قيم النفاذية و هي: الميل و التقاطع الســـيني للكميـــة التراكمية التي نفذت في وحدة الزمن ثم تم حساب ، الفترة الزمنية التي اســـتغرقتها المـــادة الفعالة قبل النفاذ، معامل الإنتشار، معامل النفاذية، معامل التجزئة و قورنت النفاذية من خلال حساب معدل تحسن النفاذية لمختلف العينات.

لقد تبين أن زيادة نسبة التحسن كانت حسب الترتيب التالي:

Benzyl alcohol (1%) > Benzyl alcohol (1%) + 20% IPA > Benzyl alcohol (1%) + 2% IPM> Benzyl alcohol (1%) + 30% IPA

ثم تم اختبار الصيغة النهائية للهلام مع التركيز الأمثل لمُحسِّن الاختراق للتخلل من خلال جلد الخنزير. وتبين أن نفاذية حامض الفوليك جيدة من خلال جلد الخنزير مع معامل نفاذية قدره 0.005 سم / ساعة.

يتم مقارنة قيم النفاذية لهلام حامض الفوليك من خلال غشاء قشر البيض الطبيعـــي وجلـــد الخنزير في الجدول التالي :

	غشاء قشر البيض الطبيعي	وجلد الخنزير
TL [h]	1.58 h	4.78

D [cm2. h-1]	4.22 x10-5	5.89 x10-4
P	0.036	0.005
K	16.9	1.1

تم دراسة ثباتية هلام حامض الفوليك من خلال تخزين الجل بشكله النهائي معبأ في أنابيب الألومنيوم علي ظروف تخزين مختلفة مثل 25 ± 2 درجة مئوية / 60٪ ± 5٪ رطوبة نسبية ، 30 ± 2 درجة مئوية / 65٪ ± 5 و40 ± 2 درجة مئوية / 75٪ ± 5٪ رطوبة نسبية, حيث تم اختبار العينات بعد أسبوعين وستة أسابيع لمحتوى جامض الفوليك) والمظهر الجسدي والرقم الهيدروجيني واللزوجة

لقد تبين أن هلام حامض الفوليك 0.1٪ ثابت من جميع الجوانب للفترة التي تم اختبارها (6 أسابيع) في جميع ظروف التخزين.