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Review Article

PHYSICOCHEMICAL, PHARMACOLOGICAL AND ANALYTICAL PROFILE OF FOLIC ACID: A COMPREHENSIVE REVIEW

Vikas Yadav [1], Yogesh Rohilla [1], Manjusha Choudhary [2], Nitesh Choudhary [3], Vikaas Budhwar [1]*
[1]Department of Pharmaceutical Sciences, Maharishi Dayanand University, Rohtak, Haryana, India
[2]Assistant Professor, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, India
[3]Assistant Professor, R.P. Inderaprastha Institute of Technology, Gharaunda, Karnal, India
*Corresponding Author Email: vikaasbudhwar@yahoo.com

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ABSTRACT

The importance of folic acid can be felt from the fact that it is required by a female since she plans for pregnancy. It is requiring 1 to 1.5 fold by a pregnant lady that is required by an adult person. Folic acid is needed by a person daily to undergo vital body function like hindrance in cycle, synthesis of DNA, RBC production etc. Deficiency of folic acid may lead to a severe complication like neural tube defects, megaloblastic anemia, malignancy and pregnancy complications. The present review summaries the physicochemical properties, biological action, pharmacological effect, therapeutic potential and analytical methods of folic acid. The recent researches on folic acid in the pharmaceutical field have also been discussed.

Keywords: Folic acid, Folate, food fortification, folic acid degradation, Photolysis

INTRODUCTION

In 1941, folic acid derived its name from the latin word *Folium* Leaf. It was first isolated from spinach leaves where it is now known to occur in relatively minor amounts compared to other food sources. Several apparently unrelated factors have been isolated in various laboratories before realization that they have in common the same parent compound, pteroyl-L-glutamic acid: factor U (a chick growth factor), vitamin M (a factor for monkeys), vitamin Bc (a chick anti-anemia factor) and others. In 1972 The International Union of Nutritional Sciences Committee on Nomenclature decided that the term folacin should be used as the general description of folic acid. However, the USP (1990) continues to call the vitamin as pteroyl glutamic acid[1].

Folic acid is widely distributed in plants, animals and microorganisms. The vitamin is present free or combined with one or more additional molecules of L(+) glutamic acid in liver, kidney, mushrooms, spinach, yeast, green leaves and grasses and also present in many micro organisms. The highest (0.25 to 1.67 mg/ml) yield being obtained from *Bacillus subtilis, B.vilgalys, Serratia marcescens* and a gram –ve *Bacillus* from chick intestine[1].

This expressed folic acid was made out of three rings (Pteridine ring, paraminobenzoic acid and glutamic acid) and was called 'pteroylglutamic acid (PGA). Long after the synthesis of folic acid it was confirmed that pteroylglutamic acid is different from natural occurring folates in three respects: (1) extra glutamate deposits (polyglutamates), (2) decrease to di-or tetra-hydroforms, and (3) extra single carbon units, e.g. methyl ($–CH_3$), formyl (-CHO), methylene ($=CH_2$), methenyl ($=CH_4$) connected to the N_5 or N_{10} nitrogen particles. Folic acid (pteroylglutamic acid) is currently used to signify the completely oxidized chemical compound, not present in normal substances[2]. Stokstad and his associate in his institute at Berkeley in 1963 studied to separate and describe a large number of the mammalian chemicals engaged with folate digestion[2]. After chemical synthesis of folic acid in 1945 by Angier *et al*., it was largely used for treatment of all kind of megaloblastic anemia, yet prior found that it is utilized to refine liver preparations, for example the megaloblastic anemia of sprue, coeliac ailment, pregnancy and malnutrition[3,4]. It was observed that slightly curing pallor in Addisonian pernicious anaemia[5,6]. However it shows that anemia deficiency and neurological damage was not prevented by folic acid due to its precipitation by this treatment[7-11]. Table 1 enlists the landmark achievements of selected scientists in the discovery & revelation of facts & information about folic acid.

Table 1: Landmark Research about Folic Acid

Years	Scientists	Discovery
1930, 1931	Wills & Mehta[12,13]	Extract of yeast is useful for prevented the anemia in rats
1931	Wills[14]	Macrocytic anemia in pregnant women is prevented by Yeast or Marmite
1932	Vaughan[15]	Marmite helps in the correction of anemia coeliac disease
1938	Wills & Evans[16]	Macrocytic anemia, nutritional and pregnancy disorder cannot be improved by liver extract
1938	Day *et al.*,[17]	Anemia in monkeys can be cured by folic acid
1938	Stokstad & Manning[18]	Vitamin B_C present in yeast, prevents
1940	Hogan & Parrott[19]	Established the fact that anemia in chicken occur due to nutrition deficiency
1940	Snell & Peterson[20]	Norit eluate factor – factor absorbed from yeast or liver is growth factor for *Lactobacillus case*

1941	Mitchell *et al.*,[21]	Compound that shown to be a growth factor for *Streptococcus lactis R* (*S. faecalis*) was named 'Folic acid'
1943	Fullerton; Watson & Castle[22]	Idiopathic steatorrhoea megaloblastic anemia could be cured from liver extracts or yeast extract
1943	Wright & Welch[23]	Folate polyglutamates is converted to monoglutamates which is microbiologically active. This all process is completed by enzyme hydrolysis.
1944	Binkley *et al.*,[24]	2 to 5 % of vitamin B complex is obtained from by yeast extract and active for *L. casei*. which need enzyme digestion to equalize activity
1945	Angier *et al.*,[25]	Folic acid was synthesized and called pteroylglutamic acid
1945	Day *et al.*,[26]	Purified *L. casei* factor is vitamin M (anemia in monkeys)
1946	Pfiffner *et al.*,[27]	Naturally occurring folate in liver is a heptaglutamate

Physicochemical properties of folic acid

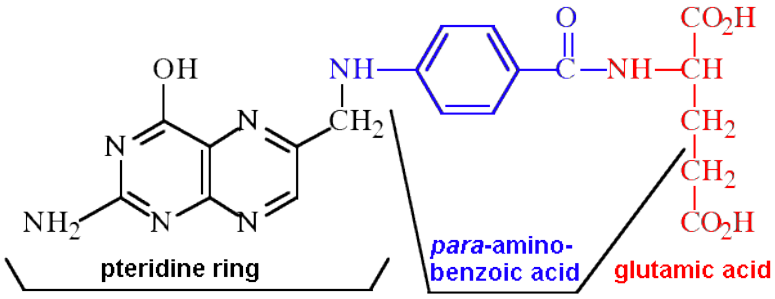

Figure 1: Folic Acid Structure

Folic acid ((S)-2-(4-((2amino-4-hydroxypteridin-6-yl) methylbanzamido) pentanedioic acid) is also known as N-[p-[[(2-amino-4- hydroxy-6-pteridinyl) methyl]- amino] benzoyl]-Lglutamic acid, vitamin B_9 & folacin. Its molecular weight is 441.403 g/mol and molecular formula is $C_{19}H_{19}N_7O_6$. Darkens and chars at about 250°C before melting. Folic acid contains three rings Pteridine, para-aminobenzoic acid (PABA) and glutamic acid. Base pteridine is connected to para-aminobenzoic acid by a methylene bridge and glutamic acid is attached to PABA by peptidic bond[28] (Fig 1). It is a yellow to orange colored, relatively odorless and present as crystalline powder. Folic acid is soluble in dilute alkaline and in dilute acidic medium. But it has low solubility in water (1.6 mg in 1000 ml at 25° C) and it is more stable in alkaline medium. It is insoluble in methanol, ethanol and dichloromethane[29]. In solution form it is easily deteriorated by light and oxidation. It is relatively soluble in acetic acid, phenol, pyridine, solutions of alkali hydroxides and carbonates. A suspension of 1 gram folic acid in 10 ml water has a pH of 4 to 4.8. Aqueous solutions prepared with sodium carbonate have the pH between 6.5 and 6.8. The pKa value of the molecules is 4.7, 6.8 and 9.0 at 30°C[30]. In amino acid solutions folic acid gets precipitated due to high concentration of calcium ions, but it is stable at pH above 5.6[31]. Table 2 enlists some of the physico-chemical properties of folic acid.

Table 2: Physico-Chemical Property

Physico-Chemical Properties of Folic Acid	
Molecular formula	$C_{19}H_{19}N_7O_6$
Molar mass	441.40 g mol−1
Appearance	yellow-orange crystalline powder
Melting point	250 °C (523 K), decomposition.
Solubility in water	1.6 mg/L (25 °C)
Acidity (pKa)[32]	1st: 4.7, 2nd: 6.8, 3rd: 9.00

Stability

The four main modes of folic acid degradation studied have been due to thermal degradation, UV radiation and oxidation. Solid folic acid is heat stable degrading between 148ºC-262ºC. It melts and decomposes rapidly starting with the loss of glutamic acid, then two overlapping reactions beginning with the loss of pterin and then p-aminobenzoic acid. Gamma-radiolysis of folic acid in aqueous solution was found to yield a mixture of pteroic acid, glutamic acid, 6-methylpteridine, para-aminobenzoic acid, and γ-aminobutyric acid. Because the salt should not be exposed to temperatures as high as 148ºC or γ-irradiation these modes of degradation are not of concerned. The most expected modes of degradation are reduction and oxidation[33,34].

Folic acid is stable in aqueous solution with citrate phosphate buffer at pH 6 to 9.8. At pH 5 to lower, it has a very low solubility and the solutions loose potency rapidly. Considerable decomposition of folic acid takes place in solutions containing riboflavin, less with thiamine HCl and practically none with pentothenyl alcohol, pyridoxine HCl and nicotinamide. However, aqueous solutions of folic acid at pH 3 and 4 are stable in presence of B-complex containing riboflavin and thiamine HCl. The decomposition of folic acid is associated with the liberation of an equivalent amount of an aromatic amine, probably p-amino benzoyl glutamic acid. It has been reported that folic acid is not stable in solution containing vitamin B complex[33,35].

The stability of folic acid to light and heat has been investigated polarographically. Aqueous solution of folic acid is exposed to light of 300-600 nm in air at various pH, folic acid decomposed rapidly to 2-amino-4-hydroxy-6-pteridylaldehyde, 2-amino-4-hydroxy-6-pteridine carboxylic acid and an unknown easily reducible compound. The velocity of decomposition is greatest at pH 3 and increases with the lowering of concentration. The unknown compound is derived by the photo-decomposition of 2-amino-4-hydroxy-6-pteridine carboxylic acid. In the alkaline solution at pH 8.8 about 10% of folic acid is decomposed into pteridine moiety and para amino benzoyl glutamic acid by heating at 100°C. The decomposition of folic acid to 2-amino-4-hydroxy-6-pteridylaldehyde is accelerated by heating in air in the presence of active carbon[36].

Thermal Property: Solid folic acid is heat stable degrading between 148ºC-262ºC[33]. Many studies on folic acid are done by different scientists who reported thermal degradation of folic acid (Fig 2). Folic acid contains three compounds, p-amino benzoic acid, pterin and glutamic acid. These three compounds were

separated from folic acid at different temperatures. Spectroscopic techniques (infrared, mass, FTIR) and X-ray diffraction technique have been used for the determination of degradation pattern of folic acid. Folic acid melts and decomposes rapidly starting with the loss of glutamic acid, then two overlapping reactions beginning with the loss of pterin and then p-aminobenzoic acid[33,34]. Tripet and Kesselring report in 1975 shows that folic acid decomposed up to 1% per year in 20°C temperature and 65% humidity. When folic acid was heated to 800°C following changes were observed. Firstly around 180 °C glutamic acid moiety was removed but pterin and PABA in overlapping manner. At 195°C amide and acid functionality were totally lost and after 200°C crystalline folic acid changed in amorphous state[37]. Jankovi also reported same degradation pattern of folic acid drug product[38].

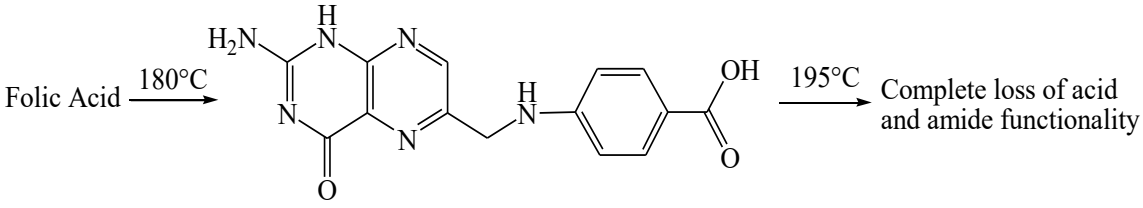

Fig 2: Thermal degradation of folic acid

Photo Stability: In 2002, Thomas *et al*., analyzed the photostablity on folic acid or other based moiety of folic acid and also analyzed the effect of pH on folic acid with light. Song and Hwang reported that folic acid absorb UV light[39].

Researchers reported that UV radiations penetrated through skin and degraded folate in human blood. Two types of radiations are UV-A and UV-B which penetrate into the dermal layer of skin hence ability of UV rays affects the folate level in the blood through indirect degradation pathway[40]. Kristian *et al.,* studied the photo degradation pattern of folic acid by UV rays. He also found that folic acid degraded by UV rays and also reported the photo-degradation phases of folic acid[41]**.** In 1949 Lowry et al. investigated the photoproducts generated as a result of photo-degradation of folic acid when it was exposed to UV light. Degradation of folic acid happened through breakdown on the methylene bridge into 6-formylpterin (FPT). On further exposure to UV light corresponding carboxylic acid derivatives were formed and finally ended into the formation of decarboxylated 2-amino-4-hydroxypteridine (Fig. 3). Further degradation of these compounds can lead to the formation of pterin or cleavage of PGA to PABA and Glutamic acid[42].

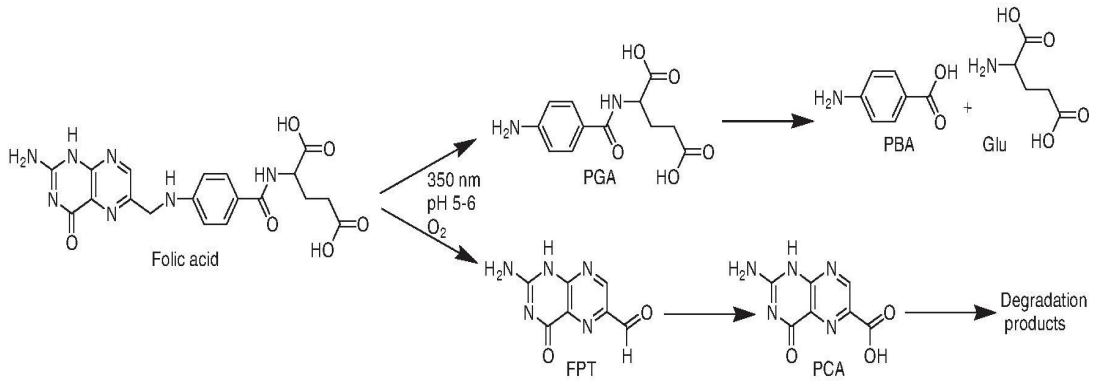

Fig 3: Degradation pattern of folic acid by UV light

Oxidation: In 1983, Day and Gregory reported that folic acid stability is affected by oxygen. The high level of oxygen level decreases the concentration of folic acid. Hence, a controlled environment is required to prevent degradation of folic acid by involving nitrogen instead of oxygen[43]**.**

Thomas *et al.,* identified the effect of oxygen on degradation of folic acid, FPT (6 formylpterin); it is the first degraded product of folic acid. Further FPT was oxidized to PCA (pterin-6-carboxylic acid) and without the presence of oxygen formation of red compound was formed which absorbed at 480 nm. Reoxygenation of the latter solution which leads to the formation of PCA (6 formylterin) and it is thought that the red-colored compound is (6-carboxy-5,8-dihydropterin) (Fig. 4). Thomas *et al*., found that the degradation of folic acid takes place with the consumption of oxygen. When 6-formylpterin degrades to PCA, it consumed low oxygen[39,44].

Liang *et al*., studied that by the addition of an antioxidant (Vitamin C) the stability of folic acid increased with the consumption of oxygen decreased. When folic acid is stored separately it completely degraded in 12 days but it stored with antioxidant it degrade only 8% [39.] The degradation of folic acid with light and oxygen is shown[45] (Fig 4).

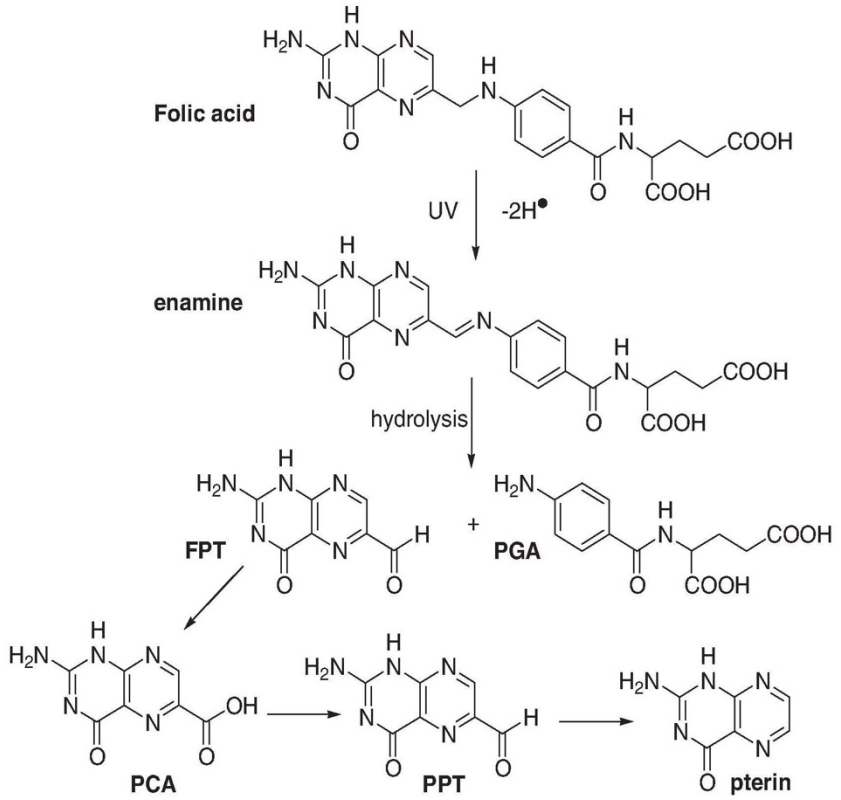

Fig 4: Effects of oxygen and UV on folic acid

Pharmacology

Biochemically folic acid is found to be in inactive form and it is converted into tetrahydro folic acid and methyltetrahydrofolate by the dihydrofolate reductase enzyme. Folic acid reached across the cells by receptor mediated endocytosis for normal function of erythropoiesis, interconvert amino acids, methylated tRNA, generate and synthesize purine or thymidylate nucleic acids. Vitamin B_{12} acts as a cofactor with folic acid for decreasing the high level of homocysteine by remethylation of homocysteine to methionine by means of methionine synthetase[46,47].

Physiological significance of folic acid

Folic acid has various functions in the body. It aids in for normal functioning of brain, helps in the formation of DNA and RNA, a responsible factor for the growth of tissues and cells, helpful during pregnancy and adolescence etc. Both folic acid and vitamin B_{12} are responsible factors for the production of RBC_C and also help in the proper functioning of iron in the body. Amino acid homocysteine level in blood is maintained by the folic acid, vitamin B_6 and B_{12}. The level of homocysteine is higher in the patients suffering from heart diseases. However, whether homocysteine causes heart diseases or is yet not explored[47].

Folic acid plays an important role in for cell development, cell growth and development through many reactions including histidine cycle, serine and glycine cycle, methionine cycle, thymidylate cycle, and purine cycle. Since after the deficiency of folic acid in the body it effects the normal functioning of body and causes various problems like megaloblastic anemia, malignancy and neural tube defects[48].

Histidine: This cycle includes the deamination of histidine in the presence of folic acid, due to production of urocanic acid. Urocanic acid is associated with numerous metabolic reactions to produce formiminoglutamate which called "FIGLU" and in the presence of formiminotransferase enzyme glutamate is synthesized. Folic acid deficiency in the body results in improper catabolism of FIGLU leading to an obstruction in the synthesis of glutamate from formiminoglutamate. So formiminoglutamate deposit in the blood and excreted through the urine[49]. Glutamate is most important factor for metabolism of sugar and fats, take part in the process of transporting the potassium ion to the spinal fluid or across the blood brain barrier[50].

Serine and Glycine Cycle: Serine is a non-essential amino acid which can be obtained from glucose or from the daily routine food. Kidney and some other tissues are responsible for glycine production. Both serine and glycine are transported rapidly through mitochondrial membrane[51]. Folate plays an important role in this pathway; 5,10methylene tetrahydrofolate gives a hydroxymethyl group to glycine residue and is used for the formation of serine which gives one carbon unit that is utilized as a part of folate reactions[52]. Without the folic acid, the glycine cannot synthesize serine which is needed for proper function of CNS. Other implication includes the obstruction of other body function like RNA and DNA synthesis, formation of muscle and metabolism of fatty acid[53].

Methionine Cycle: Folate plays an important part in the methionine cycle. In the process of methylation, 5-methyl tetrahydrofolate methionine is involved, and one methyl group takes part in homocysteine, then formation of methionine with the help of methionine synthase enzyme. Methionine synthase enzyme is dependent on the vitamin B_{12} and folic acid[54]. Deficiency of folic acid in the body effects synthesis of methionine which causes various problems like in the production of glutathione (antioxidant) and amino acid (taurine and cystine) which are helpful in removing toxins from the body[55].

Thymidylate Cycle: Thymidylate synthase helps in the reproduction of cells and tissues[21]. Antagonist of Folate inhibits this enzyme therefore have been used as anticancer agents. From this cycle, the role of folate can be linked to cancer. Thymidylate synthase shows metabolic toxic effects that can cause folate deficiency due to which body's cells develop quickly and synthesis of DNA increases. That is the reason, folate is known as an "anticancer agent"[56].

Purine Cycle: Derivatives of tetrahydrofolate are used for de novo biosynthesis of purine; folate is also used for the determination of carbon position (C2 and C8) in the purine structure. Purine has important function in the body like growth of cells; its division and improvement, since it is thought to be along with the pyrimidine base of the DNA helix. Deficiency of folate in the body causes various types of problems like defects in the DNA synthesis, which effects each part of the body such as skins, muscles, causes cancer, coordination in memory, heart and muscles diseases etc[57,58].

Clinical Significance[59]

1. Folic acid donates methyl group and aids in many vital bodily functions like DNA synthesis.
2. It plays a therapeutically important role to maintain homocysteine levels thereby preventing neural tube defects.
3. It may have to be crucial to prevent cervical dysplasia and protect neoplasia in ulcerative colitis.
4. Folic acid also promises to be a vital nutrient in treating vitiligo and may reduce inflammation of the gingiva.
5. Foliate deficiency may lead to certain neurological, cognitive, psychiatric peripheral neuropathy, insomnia, endogenous depression, myelopathy, irritability, restless legs syndrome, dementia, forgetfulness, organic psychosis and schizophrenia-like syndromes.

Pharmacokinetics

Absorption and Distribution

Folic acid belongs to BCS class II. It is rapidly absorbed through the proximal part of small intestine, from the foodstuffs. Before being absorbed it gets reduced into folylpolyglutamates. After being absorbed it get converted to tetrahydrofolate which finally conjugates with cells to make active polyglutamates. This N5 – methyl-H4 folate binds with plasma protein in the portal circulation. The commercially available crystalline folic acid is better absorption than the dietary folates. It takes about 30-60 min to attain its C_{max}. However, the cellular effects of folic acid are indifferent to its C_{max}. Folic acid is principally stored in the liver and cerebrospinal fluid. The breast milk also contains folate[60,61].

Metabolism and Elimination

Folic acid is primarily metabolized in the liver in the presence of ascorbic acid and into tetrahydro folic acid by dihydrofolate reductase enzyme. Its small proportion is also metabolized in plasma by the same pathway. Its deficiency can cause scurvy. Kidney is the main sight of elimination of folic acid. Its small proportion also undergoes entero hepatic circulation. About 4-5 mg of folic acid is excreted through urine, predominantly after its metabolism, excess quantity is excreted by the kidney. This means that its renal secretion increases with the proportional increases in its dose. As it is removed by dialysis, the patients on dialysis require an additional intake of folic acid 100-300 mg[60,61].

Patho-physiology

Dosage: The dosage of folic acid depends upon the clinical condition of the patient. A minimum dosage of 800 mcg is given to for lowering homocysteine. 1-5 mg is the dose which is most commonly given. However, to treat the conditions like cervical dysplasia, dosage > 10 mg are used (Table 3). Usual strength of folic acid available is even greater[59].

Table 3: The daily recommendations dose for dietary folic acid[62]

Infants: 0-6 month	50-65mcg
7-12 month	70-80 mcg
Children: 1-3 month	120-150 mcg
4-8 years	150-200 mcg
14<18 years	400 mcg
Pregnant women	600 mcg
Breast feeding	500 mcg

Toxicity

Toxicity symptoms of folic acid due to high doses (more than 1000 mcg) causes gastrointestinal and nervous. It includes anorexia, nausea, abdominal distension and discomfort, flatulence, sleep disturbances, vivid dreaming, irritability, excitability, rash, broncho spasm and over activity. EEG changes and convulsions are reported with intravenous therapy of folic acid[63].

Drug-Interactions: Folic acid interacts with many drugs some of the reported interaction of folic acid with other drugs is mentioned below:

1. Antacids: folic acid and antacid should not be taken concurrently as antacids have found to interfere with absorption of folic acid through GIT[64].
2. The assay antibiotics may give false low limits by interfering with the microbiological assays for serum and folic acid concentration. Precaution should be taken while concurrently administrating folic acid with chloramphenicol as it may[65].
3. Concurrent administrating of folic acid and cholestyramine may reduce the absorption of folic acid through the GIT. Restoring by cholestyramine should be administered either 1 hour after folic acid supplements should be given 4-6 hours after its administration[66].
4. Estrogen or oral contraceptive may increase the requirement of folic acid in the body[67].
5. However, some studies do not shows any significant interaction of zinc with folic acid, some studies suggest that they should not be given concurrently because folic acid might reduce the absorption of zinc from GIT[68,69].

Analysis

Identification of Folic Acid

1. Absorbance of folic acid is examined in the range between 230 to 380 nm by UV visible spectrometer. Three maxima absorbance peaks are identified at about 256 nm (0.59), 283 nm (0.575) and 365 nm (0.206) in 0.1M sodium hydroxide solution[29].
2. Thin layer chromatographic analytical method as employed with plates coated with silica gel G and ethanol, strong ammonia and 1- propanol in ratio (60:20:20) as mobile phase. Reference solution is prepared from methanol and strong ammonia solution. A 2µl solution is applied on the TLC plate and the plate is allowed to dry. After the development of plate, it is observed under the UV light at 365 nm. Test chromatogram is compared with the reference chromatogram[29].

3. According to the USP identification test of folic acid is performed by UV method. Firstly 10 µg solution of folic acid in sodium hydroxide is prepared and examined at 256/356 nm, absorbance of folic acid is present in the range of 2.80 to 3.00[70].

Assay

1. The USP (2007) commends the assay of folic acid to be done using Liquid chromatography equipped with 280 nm detector and a 15 cm x 3.9 mm column using 2 gm of monobasic potassium phosphate dissolved in about 650 ml of water containing 12 ml tetrabutylammonium hydroxide and methanol in ratio 1:4. 3N phosphoric acid or 6N ammonium hydroxide is used for adjusting the pH at 7.0 for the preparation of mobile phase. 12 mg of folic acid is dissolved in 50 ml of ammonium hydroxide as reference solution. Solution is examined at 280 nm. Reported R_f value in USP (2007) is not less than 3.6 and relative standard is not more than 2%[70].
2. IP (2007) gives a derivatization method of the folic acid assay which includes following steps: sample is dissolved in sodium hydroxide and then further (2M) HCL, zinc powder, sodium nitrite, ammonium sulphamate is added step wise. The prepared solution gives absorption maxima at 550 nm[29].
3. The BP (2009) specifies that the assay of folic acid by Liquid chromatography equipped with spherical octylsilyl silica gel (5µm) and the test solution is prepared by the sodium carbonate, diluted with mobile phase (methanol, potassium hydroxide phosphate and dipotassium hydrogen phosphate). Reference solution is also prepared in the same way. The prepared solution is examined at 280nm. Folic acid retention time is observed at 8.5 min[71].

Differential Scanning Calorimetry (DSC): Vora *et al.,* 2004 reported from the DSC curve that folic acid doesn't have an observed melting point, as it rapidly decomposes before melting with three overlapping endothermic peaks evident in (Fig 5). For the degradation of (2mg) folic acid total heat required is 960±24 $J.g^{-1}$ from 148– 262 °C (Fig 5). The first reaction shows response at 40% of the total reaction which represent loss of glutamic acid with liberation of two molecules of water. Final reaction accounts for the loss of pterin and para amino benzoic acid. DSC were attributed to the loss of glutamic acid moiety and its respective degradation at around 180°C along with an endothermic peak at 250°C as observed in DSC and IR spectra[33].

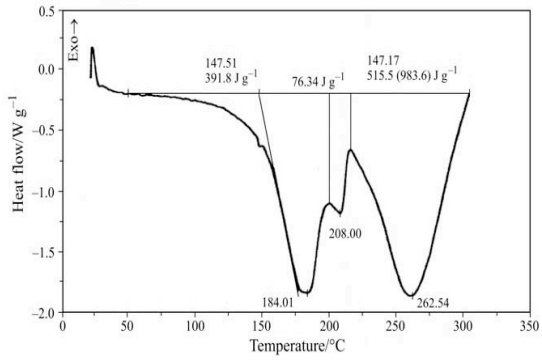

Fig 5: DSC

Thermo-Gravimetric Examination: Vora *et al*., 2004 obtained TG/DTG plot of folic acid using established optimum conditions at heating rate of 10°C min^{-1} and flow rate of dry nitrogen gas (100 ml min^{-1}). It was observed from the plot that the mass of the folic acid degrades at 100°C with the loss of water. The completion of decomposition reaction shows the complete loss of the mass of folic acid. The TG/DTG curve shows the four stages and almost all stages are shown (Fig 6) [33,72] .

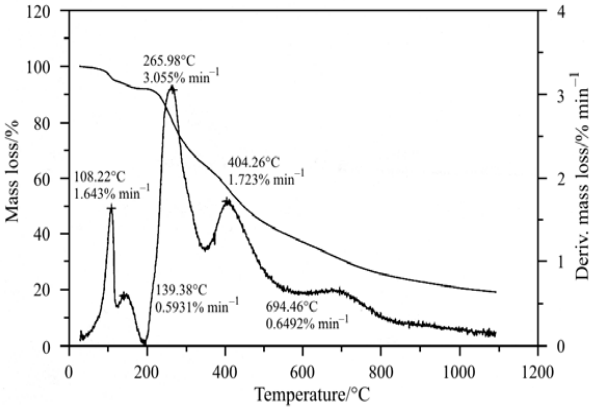

Fig: 6 TGA

X-Ray Powder Diffraction (XRD): The data, of X-ray diffraction pattern of folic acid at 349°C, indicates the formation of an amorphous product with the loss of absorbed water, with an amorphous anhydrate or lower carbonate. Followed by the decomposition of the amorphous compound a comparison graph plotted between the folic acid and the residue, left in the tube furnace at 349°C in the amorphous form and completely change the crystalline character of the product (Fig 7). Carbon showed the almost similar pattern to the standard pattern and result showed that residue was carbon. XRD is useful to evaluate the decomposition product of the folic acid and to identify its characteristic features[33].

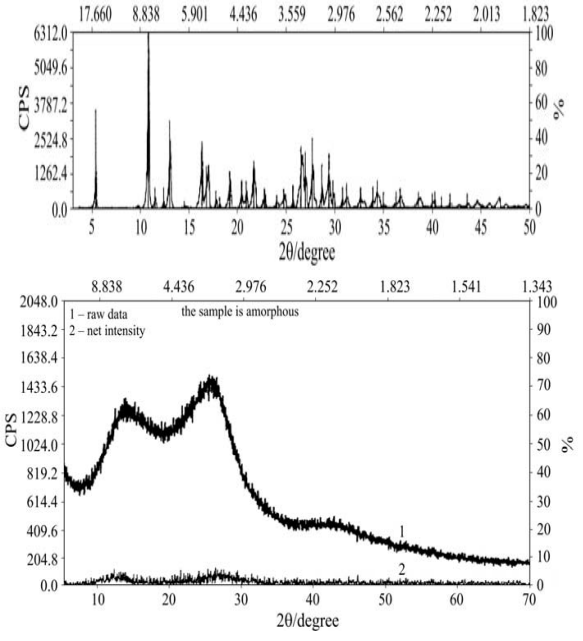

Fig 7: XRD

Determination of folic acid by spectroscopy technique

Fourier-Transform Infrared Spectroscopy (FTIR): The FTIR spectrum of folic acid shows the carbonyl absorbance at 1689 cm^{-1} due to (–CONH–) and (–COO–) groups, respectively. However the –CONH– absorbance seems a bit stronger than the (–COO) absorbance. In addition, the bands between 3000–3700 of both conjugates belong to the amine (–NH2) and amide (–CO– NH–)

stretches of folate. Additionally, the bands below 1700 correspond to the out-of plane and in plane motions of (–NH2) and (C–N=) stretches of folic acid[73].

The infrared spectra gave a definitive pathway to the degradation of folic acid. As found, in the IR spectra of folic acid at room temperature (Fig 8a), the different functional group can be observed. Spectra compared with sample pretreated at 140° C. The main preceding range observed for examination of functional group is 3000-1500 cm^{-1} and other main region observed is 900-700 cm^{-1} which represents the bending region of functional group (Fig 8b). Both symmetric as well as asymmetric CH stretch is present in absorption spectra region 2960-2860 cm^{-1}. C=O group present in absorption region of 1860-1540 cm^{-1}. The C=O group at 140° C functionality at 1780 cm^{-1} showed acid function remove which indicated the loss of acid moiety. The functional group at 180° C in the 1680-1650 cm^{-1} region showed that amide group loss which indicated the formation of strong amide bond. At 195°C no group were observed which shows that folic acid was completely burn at this temperature (Fig 8c). Due to CO_2, abnormal peaks obtained in region at (1000 cm^{-1}) [33,74].

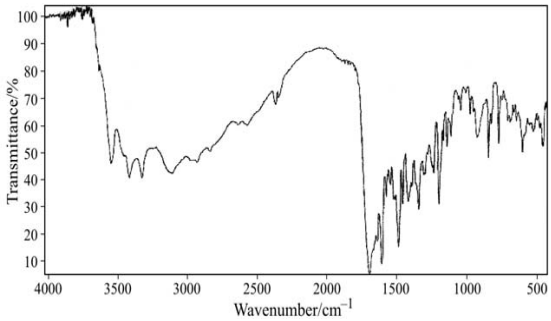

Fig: 8a FTIR at room temperature (28°)

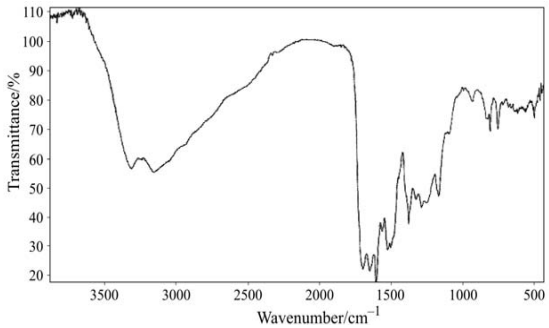

Fig: 8b FTIR at 140°C

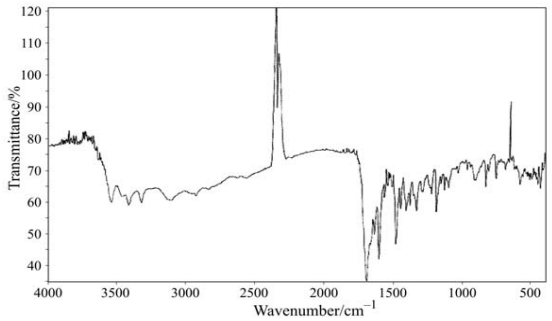

Fig 8c: FTIR at 195°C

Mass Spectroscopy: Recently electrons spray ionization technique is used for mass spectroscopy. Mass analyzer detected on the basis of mass to charge ratio (m/z) for analyte and ionization sample, folic acid (m/z 442) and 5-MTHF (m/z 460), ionization of sample at 447 and 465. First fragment peak of folic acid and 5-MTHF observed to be 295 and 313 as shown (Fig 9). It showed the loss of glutamic acid moiety. The Collision-induced dissociation showed the loss of 13C from the glutamic acid. For ionization sample fragment peak of folic acid and 13C5 observed to be 442-295, 447-295, 460-313 m/z and 5-MTHF, respectively[75].

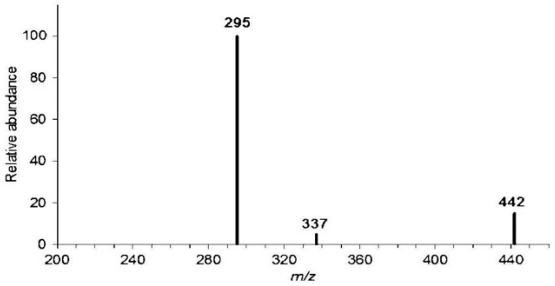

Fig 9: Mass spectroscopy

Determination of folic acid by chromatography technique

HPLC: Firstly prepared sample is separated by the reverse phase liquid chromatography from column 18. Preparation of mobile phase of pH 5.5 by using 40 mM sodium phosphate dibasic, heptahydrate and 8 % acetonitrile in ratio (80:12:8) and after that pH (5.5) adjusts from 85% phosphoric acid. Before the use of mobile phase, it is filtered from the 0.2 μm filter and the gases are removed. The flow rate is maintained at 0.9 ml/min and temperature of column is also maintained at 25°C.The HPLC unit works on a constant flow rate. Distribution of folate can be analyzed by the peak area under the electrochemical signal at porous graphite electrode. High potential is applied for achieving greater sensitivity and stability of baseline. Compounds which are electro active like folate, they oxidised at a specific point because every compound has different oxidation potential. Compounds that completely oxidize at lower potential cannot be detected at higher potential. Lebiedzinska A *et al.,* has given an HPLC method to represent the folic acid from fortified food [76,77].

TLC: Firstly, TLC plate of fluorescent silica gel is prepared by developing the plate in ethyl acetate solution and drying. The standard reference spot is applied at 2 cm with 10 µl solution & then the chromatogram is developed in ammonium hydroxide and propanol in the ratio (1:3). Plate are developed and & left for drying. Then the plates are scraped separately, and the material is transferred in test tube, to make the solution in dibasic potassium phosphate and centrifuge for 10 min at 1000 rpm. Then folic acid is extracted from the silica gel and is examined at 550 nm. The R_f value of folic acid is found to be 0.50 from the other impurities at R_f (0.28, 0.66, 0.74 and 0.80)[78].

CONCLUSION

Folic acid was perhaps being utilized by living being since the life originated on the earth. However, it's important was realized after its discovery by Lucy Wills in the year 1931. Since then constant efforts were played by the scientist and researcher to analyze this molecule in different food stuffs. Qualitatively and quantitatively, enhance its pharmaceutical characteristics like aqueous solubility and shelf life. Ongoing efforts in this regard the future era wood

witness better formulation of folic acid that would exploit its uses in a better way.

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REFERENCES

1. Hazra A, Tripathi SK. Folic acid revisited. Indian Journal of Pharmacology 2001; 33:322–42.
2. Stokstad ELR. Early work with folic acid. Federation Proceedings 1979; 38, 2696–98.
3. Hanes FM. Diagnostic criteria and resistance to therapy in the sprue syndrome. American Journal of Medical Science 1942; 204:436–43.
4. Davidson LSP, Girdwood RH, Innes EM. Folic acid in treatment of sprue syndrome. Lancet 1947; (1): 511–15.
5. Moore CV, Bierbaum OS, Welch AD, Wright LD. The activity of synthetic Lactobacillus carei factor 'folic acid' as an ant pernicious anemia substance. I. Observations on four patients, two with Addisonian perivirus anemia, one with non tropical sprue and one with pernicious anemia of pregnancy. Journal of Laboratory and Clinical Medicine 1945; 30:1056–69.
6. Vilter RW, Spies TD, Koch MV. Further studies on folic acid in the treatment of macrocytic anemia. 5th Medical Journal (Nashville) 1945; 38:781–85.
7. Amill LA & Wright M. Synthetic folic acid therapy in pernicious anemia. Journal of American Medical Association 1946; 131:1201–07.
8. Vilter CF, Vilter RW, Spies TD. Occurrence of combined system disease in persons with pernicious anemia during treatment with Lactobacillus casei factor ('folic acid'). Proceedings of the Central Society of Clinical Research 1946; 19:609–63.
9. Lavoie A, Tripp E, Hoffbrand AV. The effect of vitamin B12 deficiency on methylfolate metabolism and pteroylpoly-glutamate synthesis in human cells. Clinical Science and Molecular Medicine 1947; 47:617–30.
10. Heinle RW, Welch AD. Folic acid in pernicious anaemia: failure to prevent neurologic relapse. Journal of the American Medical Association 1947; 133:739–41.
11. Meyer LM. Folic acid in the treatment of pernicious anemia. Blood. 1947; 2:50–62.
12. Wills L. & Mehta MM. Studies in 'pernicious anemia' of pregnancy, I. preliminary report. Indian Journal of Medical Research 1930; 17:777–92.
13. Wills L. & Mehta MM. Studies in 'pernicious anemia' of pregnancy, Part IV. The production of pernicious anemia (Bartonella anemia) in intact albino rats by deficient feeding. Indian Journal of Medical Research 1931; 18: 663–83.
14. Wills L. Treatment of 'pernicious anaemia of pregnancy' and 'tropical anaemia' with special reference to yeast extract as curative agent. British Medical Journal 1931; 1:1059–64.
15. Vaughan J. Tropical macrocytic anaemia. In: Proceedings of the Royal Society of Medicine, (Discussion of Paper by L. Wills) 1932; 25:122.
16. Wills L & Evans BDF. Tropical macrocytic anaemia: its relation to pernicious anaemia. Lancet 1938; 416–421
17. Day PL, Mimms V, Totter JR, Stokstad ELR, Hutchings BL, Sloan NH. The successful treatment of vitamin M deficiency in the monkey with highly purified L. casei factor. Journal of Biological Chemistry 1938; 157: 423–424.
18. Stokstad ELR & Manning PDV. Evidence of a new growth factor required by chicks. Journal of Biological Chemistry 1938; 125:687–697.
19. Hogan AG & Parrott EM. Anaemia in chicks caused by a vitamin deficiency. Journal of Biological Chemistry 1940; 132:507–517.
20. Snell EE & Peterson WH. Growth factors for bacteria. X. Additional factors required by certain lactic acid bacteria. Journal of Bacteriology 1940; 39: 273–85.
21. Mitchell HK, Snell EE, Williams RJ. The Concentration of Folic Acid. Journal of the American Chemical Society 1941; 63:2284.
22. Fullerton HW. Macrocytic anaemia of pregnancy and the puerperium. Journal of British Medical 1943; 1: 158–60.
23. Wright LD & Welch AD. The production of folic acid by rat liver in vitro. Science 1943; 98: 179–82.
24. Binkley SB, Bird OD, Bloom ES, Brown RA, Calkins DG, Camnpbell CJ *et al*. On the vitamin B complex conjugate in yeast. Science 1944; 100:36–37.
25. Angier RB, Boothe JH, Hutchings BL, Mowat JH, Semb J, Stokstad ELR, *et al.,* Synthesis of a Compound Identical with the *L. casei* factor isolated from liver. Science 1945; 102:227–28.
26. Day PL, Mims V, Totter JR, Stokstad ELR, Hutchings BL, Sloan NH. The successful treatment of vitamin M deficiency in the monkey with highly purified L. casei factor. Journal of Biological Chemistry 1945; 157: 423–24.
27. Pfiffner JJ, Calkins DG, Bloom ES, O'Dell BL. On the peptide nature of vitamin B complex conjugate from yeast (Letter). Journal of the American Chemical Society 1946; 68: 1392.
28. United States Pharmacopoeia Dispensing Information. Vol 1: Drug Information for the Health Care Professional. 16th ed. Rockville, Maryland: United States Pharmacopoeial Convention Inc. 1998.
29. Pharmacoepeia of India, vol. Controller of Publication, Ministry of Health Department, Government of India, New Delhi. 2007 pp. 525-26.
30. Moffat AC, Blair IA. Clerk's Isolation and Identification of drug (2nd edition). Journal of Pharmacy and Pharmacology 1986; 38(12):942-44.
31. Gazzali AM, Lobry M, Colombeau L, Acherar S, Azaïs H, Mordon S, *et al.,* Stability of folic acid under several parameters. European Journal of Pharmaceutical Sciences 2016; 93:419–30.https://doi.org/10.1016/j.ejps.2016.08.045
32. Dawson RMC, Data for Biochemical Research, Oxford University Press, Oxford, 1989, 3rd Edition, p. 134 El-Dessouky MM, Abd-Elwahab BM and Turk SA, Effect of gamma radiation on folic acid and its cobalt complex solutions. Journal of Radioanalytical and. Nuclear Chemistry 1988, 125(2).
33. Vora A, Riga A, Dollimore D, Alexander K.. Thermal stability of folic acid in the solid-state. Journal of Thermal analysis and Calorimetery. 2004; 75:709–17.
34. El-Dessouky MM, Abd-Elvahab BM, Turk SA. Effetcs of gamma radiation on folic acid and its cobalt complex solutions. Journal of radioanalytical and nuclear chemistry 1988;125(2):255-63.
35. Vora A, Riga A, Alexander K. Processes to identify the degradation mechanism of a solid which appears to undergo a complex reaction : folic acid. Instrumentation science & technology 2002; 30(2):193-203 https://doi.org/10.1081/CI-120003899
36. Gazzali AM, Lobry M, Colombeau L, Acherar S, Azaïs H, Mordon S *et al.,* Stability of folic acid under several parameters. European Journal of PharmaceuticalSciences 2016; 93:419–430. https://doi.org/10.1016/j.ejps.2016.08.045

37. Tripet FY, Kesselring UW. Vitamin in Pharmaceutical Formulation. Pharmaceutica Acta Helvetiae 1975;50:318.
38. Jankovi B. Thermal stability investigation and the kinetic study of Folnak® degra- dation process under nonisothermal conditions. Journal of the American Association of Pharmaceutical Scientists 2009; 11:103-112.
39. Thomas AH, Lorente C, Capparelli AL, Pokhrel MR, Braun AM, Oliveros E. Fluorescence of pterin, 6-formylpterin, 6-carboxypterin and folic acid in aqueous solution: pH effects. Photochemical and Photobiological Science 2002; 1, 421–426.
40. Borradale DC, Kimlin MG. Folate degradation due to ultraviolet radiation: possible implications for human health and nutrition. Nutrition Reviews. 2012; 70: 414–422.
41. Kristian M, Engeset A, Carmen A, Juzeniene A, Vorobey A, Johnsson A, Moan J. Ultraviolet photodegradation of folic acid. Journal of Photochemistry and Photobiology B: Biology 2005;80:47–55.
42. Lowry OH, Bessey OA, Crawford EJ. Photolytic and enzymatic transformations of pteroylglutamic acid. Journal of Biological Chemistry 1949; 180: 389–398.
43. Day BPF, Gregory III JF. Thermal stability of folic acid and 5- methyltetrahydrofolic acid in liquid model food systems. Journal of Food Science 1983; 48: 581–587.
44. Thomas AH, Suarez G, Cabrerizo FM, Martino R, Capparelli AL. Study of the photolysis of folic acid and 6-formylpterin in acid aqueous solutions. Journal of Photochemistry and Photobiology A: Chemistry 2000; 135:147–154
45. Liang XS, Zhao FQ, Hao LX. Research on stability of synthetic folic acid. Advance Materials Ressearch 2013; 64:781-784, 1215–1218.
46. Krebs MO, Bellon A, Mainguy G, Jay TM, Frieling H. One-carbon metabolism and schizophrenia: Current challenges and future directions. Trends in Molecular Medicine 2009;15:562-70.
47. Mahmood L. The metabolic processes of folic acid and Vitamin B_{12} deficiency. Journal of Health Research and Reviews 2014;1:5-9.
48. Gropper SS, Smith JL. Advanced Nutrition and Human Metabolism. United States: Cengage Learning 2005. p. 371.
49. García-Miss Mdel R, Pérez-Mutul J, López -Canul B, Solís-Rodríguez F, Puga-Machado L, Oxté-Cabrera A *et al*., Folate, homocysteine, interleukin-6, and tumor necrosis factor alfa levels, but not the methylenetetrahydrofolate reductase C677T polymorphism, are risk factors for schizophrenia. Journal of Psychiatric Research 2010;44:441-46.
50. Bhagavan V. Medical Biochemistry. United Kingdom: Academic Press 2002. p. 521-46.
51. Allen RH, Stabler SP, Savage DG, Lindenbaum J. Diagnosis of cobalamin deficiency I: Usefulness of serum methylmalonic acid and total homocysteine concentrations. American Journal of Hematology 1990;34:90-98.
52. Ulrich CM. Nutrigenetics in cancer research-folate metabolism and colorectal cancer. The Journal of Nutrition 2005; 135:2698-702.
53. Varela-Moreiras G, Murphy MM, Scott JM. Cobalamin, folic acid, and homocysteine. Nutrition Reviews 1990; 67:S69-72.
54. Dietrich M, Brown CJ, Block G. The effect of folate fortification of cereal- grain products on blood folate status, dietary folate intake, and dietary folate sources among adult non-supplement users in the United States. The Journal of American College of Nutrition 2005; 24:266-74.
55. Cabanillas M, Moya Chimenti E, González Candela C, Loria Kohen V, Dassen C, Lajo T. Usefulness of meal replacement: Analysis of the principal meal replacement products commercialised in Spain. Nutrition Hospitalaria 2009;24:535-42.
56. Jia ZL, Li Y, Chen CH, Li S, Wang Y, Zheng Q, *et al*., Association among polymorphisms at MYH9, environmental factors, and nonsyndromic orofacial cleft s in western China. DNA and Cell Biology 2010;29:25-32.
57. Bazzano LA. Folic acid supplementation and cardiovascular disease: The state of the art. American Journal of Medical Sciences 2009;338:48-9.
58. French AE, Grant R, Weitzman S, Ray JG, Vermeulen MJ, Sung L, *et al.,* Folic acid food fortification is associated with a decline in neuroblastoma. Clinical Pharmacology & Therapeutics 2003;74:288-94.
59. Lambie DG, Johnson RH. Drugs and folate metabolism. Drugs. 1985; 30:145-55.
60. United States Pharmacopoeia Dispensing Information. Vol 1: Drug Information for the Health Care Professional. 16th edition. Rockville, Maryland: United States Pharmacopoeial Convention Inc 1998.
61. Folic acid. In: Reynolds JEF, Parfitt K, Parsons AV, Sweetman SC, editors. Martindale: The Extra Pharmacopoeia. 31st ed. London: The Royal Pharmaceutical Society 1996:1361-2
62. Auerhahn C. Daily folic acid supplementation for 3 years reduced age related hearing loss. Evidence Based Nursing 2007;10:88.
63. Butterworth CE, Tamura T. Folic acid safety and toxicity : a brief review.American society for clinical nutrition 1989; 50:353-58.
64. Russell R, Golner B, Krasinski S. Effect of antacid and H_2 receptor antagonists on the intestinal absorption of folic acid. Journal of Laboratory and Clinical Medicine 1984; 112:458-63.
65. McEvoy GK, editor. AHFS Drug information 90. Bethesda, MD: American Society of Hospital Pharmacists; 1990. p. 2111-2.
66. British National Formulary. Royal Pharmaceutical Society, United Kingdom, Appendix 1: Drug interactions 1998; 35:565.
67. D'Arcy P. Drug interactions with oral contraceptives, Annals of pharmacotherapy 1986; 20(5):353-62.
68. Milne D, Canfield W, Mahalko J, Sandstead H. Effect oforal folic acid supplements on zinc, copper, and iron absorption and excretion American Journal Clinical and Nutrition 1984; 39:535-9.
69. Butterworth CE, Hatch K, Cole P, Sauberlich HE, Tamura T, Cornwell PE. Zinc concentration in plasma and erythrocytes of subjects receiving folic acid supplementation, American Journal of Clinical and Nutrition 1988; 47:484-6.
70. United States Pharmacopoeia Dispensing Information. Vol 1: Drug Information for the Health Care Professional. 16th ed. Rockville, Maryland: United States Pharmacopoeial Convention Inc.; 2007.
71. British Pharmacopoeia Commission. British Pharmacopoeia. London: TSO; 2009.
72. Keattch CJ and Dollimore D. An Introduction to Thermogravimetry, 2nd Ed., Heyden London; 1975, p. 28.
73. Mansoori GA, Kenneth SB, Zadeh AS. A Comparative Study of Two Folate-Conjugated Gold Nanoparticles for Cancer Nanotechnology Applications, Cancers 2010; *2*: 1911-1928.
74. Jerzy M, Zygmunt M. Analytical Chemistry, Vol 1. PWN Scientific Publisher; 2012.
75. Maria V. Chandra-Hioe & Martin P. Bucknall &Jayashree Arcot. Folate analysis in foods by UPLC-MS/MS: development and validation of a novel, high throughput quantitative assay; folate levels determined in Australian fortified breads. Analytical and Bioanalytical Chemistry. 2011; 401:1035–42. DOI 10.1007/s00216-011-5156-3.
76. Flangan RJ, Perrett D, Whelpton R. Electrochemical Detection in HPLC. Analysis of drugs and poisons (RSC

Chromatography Monographs), Royal Society of Chemistry, Cambridge 2005: p 1-5.
77. Lebiedzinska A, Dabrowska M, Szefer P. High-Performance Liquid Chromatography Method for the Determination of Folic Acid in Fortified Food Products. Journal of Toxicology Mechanisms and Methods 2008; 18:463–67.
78. Reifx vand, Reamer JT, Grady LEET. Chromatographic Assays for Folic Acid. Journal of Phaemaceutical Sciences 1977; 66(8):1112-16.

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