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# Factors Affecting Folic Acid Stability in Micronutrient Fortified Corn Tortillas

Jordan S. Chapman
*Brigham Young University - Provo*

# FACTORS AFFECTING FOLIC ACID STABILITY IN MICRONUTRIENT FORTIFIED CORN TORTILLAS

by

Jordan S. Chapman

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GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

Jordan S. Chapman

This thesis has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

| | |
|---|---|
| Date | Michael L. Dunn, Chair |
| Date | Frost M. Steele |
| Date | Lynn V. Ogden |
| Date | Dennis Eggett |
| Date | N. Paul Johnston |

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| | |
|---|---|
| Date | Michael L. Dunn<br>Chair, Graduate Committee |

Accepted for the Department

| | |
|---|---|
| Date | Merrill Christensen<br>Graduate Coordinator, NDFS Department |

Accepted for the College

| | |
|---|---|
| Date | Rodney Brown<br>Dean, College of Life Sciences |

# ABSTRACT

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Jordan S. Chapman

Nutrition, Dietetics and Food Science Department

Master of Science

Folate degradation in micronutrient fortified corn masa and tortillas was evaluated using masa prepared from either nixtamalized corn flour or fresh nixtamal. A laboratory evaluation of the effects of pH, iron, and holding time at elevated temperature on folate loss in corn flour masa failed to show significant differences in any variable-treatment combination. An additional study was conducted at a commercial tortilla mill in Guadalajara, Mexico using masa prepared from fresh nixtamal. Commercial nixtamal was fortified with one of two different micronutrient premixes, containing iron, zinc, riboflavin, thiamin, niacin and either unencapsulated or lipid-encapsulated folic acid. A batch of each fortified masa and an unfortified control batch were prepared on each of two consecutive days. Folate loss in prepared masa increased with prebake masa holding time for both premixes. Encapsulated folic acid showed a significantly lower percent loss from theoretical, indicating a protective effect from the lipid coating. No significant

differences in folate levels were found between prebake masa and baked tortillas. Holding baked tortillas for up to 12 hours also had no effect on folate levels. Results indicate that added folic acid is degraded during the grinding process, as well as during pre-bake holding of masa. Native folate showed no significant loss.

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**J.S. Chapman, F.M. Steele, D. Eggett, N.P. Johnston, M.L. Dunn**

**Brigham Young University**
**Department of Nutrition, Dietetics, and Food Science**
**S-221 ESC**
**Provo, UT 84602**

**Michael L. Dunn**
**S-221 ESC**
**Provo, UT 84602**
**Phone: (801) 422-6670**
**Fax: (801) 422-0258**
**michael_dunn@byu.edu**

**Abstract**

Folate degradation in micronutrient fortified corn masa and tortillas was evaluated using masa prepared from either nixtamalized corn flour or fresh nixtamal. A laboratory evaluation of the effects of pH, iron, and holding time at elevated temperature on folate loss in corn flour masa failed to show significant differences in any variable-treatment combination. An additional study was conducted at a commercial tortilla mill in Guadalajara, Mexico using masa prepared from fresh nixtamal. Commercial nixtamal was fortified with one of two different micronutrient premixes, containing iron, zinc, riboflavin, thiamin, niacin and either unencapsulated or lipid-encapsulated folic acid. A batch of each fortified masa and an unfortified control batch were prepared on each of two consecutive days. Folate loss in prepared masa increased with prebake masa holding time for both premixes. Encapsulated folic acid showed a significantly lower percent loss from theoretical, indicating a protective effect from the lipid coating. No significant differences in folate levels were found between prebake masa and baked tortillas. Holding baked tortillas for up to 12 hours also had no effect on folate levels. Results indicate that added folic acid is degraded during the grinding process, as well as during pre-bake holding of masa. Native folate showed no significant loss.

**Introduction**

It has been well established that a low maternal intake of folate is associated with a higher risk of neural tube defects such as spina bifida and anencephaly in newborn infants (Imhoff-Kunsch et al., 2007). Because the neural tube in a developing fetus forms within the first four weeks of pregnancy, often before many women know they are pregnant, it is recommended that all women of childbearing age consume 400 – 800 ug of folic acid each day (USPSTF, 2009). The importance of folate in fetal development has become even more apparent with the recent evidence that low maternal intakes are linked to a higher incidence of congenital heart defects in infants (Ionescu-Ittu, 2009).

Multiple studies have been conducted showing that many women and children throughout Mexico are not getting the recommended level of folate. In one study of sixty lactating women sampled from across Mexico, 77% of the women had dietary folate intakes below the recommended amount (Caire-Juvera et al., 2007). Another study of 104 Mexican women from different socioeconomic status' found that fifty four percent had intakes <200 ug/day, under half of the minimum level of 400-800ug that is recommended (Bacardi-Gascon et al., 2003). In another study of 331children aged 6-14, almost 50% were found to have low to marginal serum folate levels (Monarrez-Espino et al., 2004). Rivera and Amor (2003) estimated that 10% of Mexican children between 5 and 11 years of age were deficient in folate.

Because of the importance of folate in the diet, much effort has been made to increase its intake in the Mexican population, especially among women (de Villarreal et

al., 2006; Martinez-de Villarreal et al., 2001). A large folic acid supplementation campaign conducted in 1999 in Nuevo Leon, Mexico resulted in a 50% decrease in the incidence of anencephaly and spina bifida cases after two years (de Villarreal et al., 2002). Corn tortillas are a staple in the Mexican diet and could serve as an excellent vehicle for delivering folate and other essential micronutrients to at-risk populations in Mexico (Burton et al., 2008). Among certain segments of the Mexican population, corn tortillas represent nearly 50% of individual energy intake and constitute 60-90% of cereal product intake (Villalpando 2004).

Corn tortillas are manufactured in two primary ways. The traditional method involves cooking corn with lime (calcium oxide) and steeping overnight. After discarding the alkaline cooking liquor and washing, the resulting product (nixtamal) is then ground into masa using volcanic mill stones. The masa is then formed into tortillas and baked. The other method involves the use of corn masa flour (CMF). The same nixtamalization process is used, but instead of grinding the nixtamal into masa, it is dried and finely ground into flour which can be easily distributed. The flour is then hydrated with water, formed into tortillas, and baked.

A commercial process has recently been developed (Dunn et al., 2007; Burton et al., 2008) to allow fortification of traditionally-made nixtamal tortillas with a micronutrient premix—including folic acid—in order to increase the micronutrient intake of the Mexican population. A preferred method for micronutrient incorporation is to add a powdered vitamin/mineral premix into the nixtamal feed stream as it enters the grinding stones.

While most of the fortificant vitamins were stable through the tortilla manufacturing process, research conducted in a commercial tortilleria in Mexico (Dunn et al., 2007) showed that added folic acid was unstable, resulting in significant (80%) losses compared to theoretical targets. Furthermore, these folic acid losses were observed in fortified masa samples collected prior to baking, indicating that factors other than baking were responsible for the instability.

Factors that might be contributing to folate loss in pre-bake masa include the alkaline pH and elevated masa temperature resulting from grinding. The stone milling process generates significant heat. Masa exiting the millstones in one tortilleria in Mexico was measured at 56°C (personal communication, Gaspar Flores). The hot masa is typically stacked and held in 20 – 50 kilo bundles or containers at ambient temperature under minimal light exposure until use. Masa holding times prior to baking can vary from a few minutes to several hours. Extended holding of the hot, alkaline masa may play a role in folate loss.

A formulation-driven cause for folate degradation was proposed after comparing the results of previous studies conducted at Brigham Young University. In one study that showed only moderate folate loss in micronutrient fortified corn tortillas, ferrous fumarate was used as the iron source at a level of 23.11 mg/kg masa. In a later study that showed poor folate stability, electrolytic iron was used as the iron source at twice the level (46.22 mg/kg masa) to account for its lower bioavailability (Richins et al., 2008). The increase in iron, or the change in iron source, could have led to increased oxidation of the folate (Day and Gregory, 1983).

An additional factor to consider is masa pH. Depending on the manufacturer and consumer preference, typical masa pH is slightly to moderately alkaline. Some manufacturers wash the nixtamal more extensively prior to milling. This produces tortillas with a closer to neutral pH, a milder flavor and a whiter color. Other manufacturers add additional lime to the nixtamal and/or masa, which raises the pH and produces a more yellow tortilla. Some manufacturers have begun adding acidulants to the nixtamal to reduce the pH and thereby extend the shelf-life of their tortillas (Higueraciapara and Nieblas, 1995). In the event that masa pH is a causative factor in folate degradation, the effect of these various pH treatments on folate stability in masa should be evaluated.

Given the critical role of folate in the maternal diet, and the large percentage of the traditional Mexican diet comprised of corn tortillas, an investigation of the cause of folate degradation during production of micronutrient fortified corn tortilla masa is of vital importance to the future health and well being of the Mexican population.

**Materials and Methods**

**Study design**

The work reported here covers two separate experiments. The first experiment was a split-plot fractional factorial design using CMF. This screening study was conducted in a research laboratory under controlled conditions. The fractional factorial study was followed by a step-wise evaluation of folate degradation under actual processing conditions in a commercial nixtamal tortilla mill in Guadalajara, Mexico.

**Samples for screening study**

The ADX tool in Statistical Analysis Software (SAS Institute, Cary, NC) was used to create a split plot $2^2$ x $2^{3-1}$ fractional factorial design (see Table 1). Treatment variables were: iron source, iron level, masa pH, masa holding time, and masa holding temperature. White corn masa flour (unfortified) was obtained from Lifeline Foods (No. 4450-0050 St. Joseph, MO). Either A-131 electrolytic iron (Research Products Co. Salina, KS) or ferrous fumarate (Sigma-Aldrich St. Louis, MO) was added, at the specified level, to 250g tortilla flour along with 0.4g of a vitamin/mineral premix (see Table 2) provided by DSM Nutritional Products Mexico. The flour and micronutrients were mixed using a ½-gallon (1.9 L) ceramic jar (US Stoneware, East Palestine OH) on a US Stoneware Unitized Jar Mill (model 755RMV) with 2038g blending media (US Stoneware 90% high-density alumina spheres; ½-in diameter) for 30min. The fortified flour was stored refrigerated for no more than one day before being made into masa. Four batches of masa were made, each with a different iron level and iron source. Each batch was then used for the three sub-plot factorial treatments—pH, holding time, and holding temperature—for a total of 16 samples. Each batch of fortified masa flour was also analyzed in dry form to determine starting folate levels.

For acidic masa (pH 5), 56.5g of 85°C tap water and 1ml of commercial tortilla acidulant/preservative, comprised of propionic acid, phosphoric acid, sodium propionate, dextrose, and sodium tripolyphosphate (Chem-Mex, Southgate, CA), were combined with 50g of the fortified flour in a 4 ½ quart (4.3L) planetary mixer (Model K45 Hobart MFG CO Troy, OH). For alkaline masa (pH 9), 0.089g CaO (lime) was dissolved in 57.5g of 85°C tap water and combined with 50g of the fortified flour in the planetary

mixer. Hot (85°C) tap water was used to produce a masa ball with an internal temperature of approx. 56°C, which corresponded to the temperature of masa exiting the grinding stones. Masa samples were formed into a ball, and wrapped in plastic film to avoid moisture loss, and then wrapped in aluminum foil to prevent light exposure. The 85°C samples were incubated at either 25°C or 35°C for either 30 min or 120 min to mimic possible ambient holding temperatures in a tortilleria. Following incubation, masa was analyzed for total folate content.

**Samples for Commercial Process Evaluation**

In the Guadalajara mill study, two different micronutrient premixes (Research Products Company, Salina, KS) were evaluated. One premix contained folic acid that was encapsulated with saturated fat (75% encapsulating material:25% folic acid), and the other premix contained unencapsulated folic acid. Both premixes had similar levels of folic acid (1.47 – 1.52ug folic acid/g premix) along with thiamin, riboflavin, niacin, zinc, and electrolytic iron at the same levels used in the screening study, with iron being added at the higher level. A powder dosifier manufactured by Probst (Probst S.A. de C.V., Tlalnepantla, México) was used to add each premix to the nixtamal immediately before it passed into the grinding stones. Samples from unfortified control masa, and masa prepared from each of the two premixes were collected in duplicate on each of two consecutive days; with treatment order reversed the second day. The typical procedure followed by the mill was to collect hot masa (approx. 55°C) from the grinding mill and pack it into 20 kg pails with lids. The pails were then stacked and held until use. During this study, masa samples were collected from the pails in duplicate, after 0.5hr, 1hr, 2hr,

and 4hr of sample storage on both days. These times were chosen to cover the possible range of actual storage times of masa prior to being baked into tortillas. Masa and tortilla samples were also collected from the tortilla former/extruder (prebake) and the oven exit respectively, to evaluate bake loss. Baked tortillas, sampled in duplicate on both days, were held at refrigerated and at ambient temperatures for 12 hr and compared to samples that had been frozen immediately after baking. In addition to masa and tortillas, dry corn and nixtamal samples were taken on each day to allow for an evaluation of native folate loss across the entire process. All samples were frozen and transported back to BYU, where total folate and moisture analyses were done. Previous research and literature reports indicated that folate stability is not appreciably affected by freezing and thawing (Phillips et al., 2005). Samples were thawed at 25°C for 0 – 40 minutes depending on the type of sample. Accelerated thawing at room temperature has been shown to cause insignificant loss of folates compared to slower thawing at refrigerated temperatures (Phillips et al., 2005).

**Folate Analysis**

Folates were extracted using the AOAC trienzyme extraction method (2004.05) with some modifications. Rat plasma (0.1ml) (male, non-sterile with lithium and heparin, Pel-Freez Biologicals, 36161-2, Rogers, AR) with anticoagulant factors was used as the source of the folate conjugase enzyme. Rat plasma has recently become more commonly used because it is readily available and can easily be stripped of endogenous folate using acidified charcoal (Tamura, 1998). The folic acid working standard was brought to a concentration of 2 ng/ml, rather than the 10 ng/ml concentration called for in the method.

After filtering through Whatman 2V filter paper, samples were diluted with distilled water by an appropriate amount to have the samples fall within the standard curve.

The microbiological assay (*L. casei* subsp. *Rhamnosus*, ATCC #7469) of Tamura (1990) was used with 96-well microtiter plates and minor modifications. Inoculum was maintained by weekly transfers into fresh lactobacilli broth AOAC. Prior to plating the samples, cultures were transferred to depletion media, prepared from lactobacilli broth and folic acid casei media per the method of Chen and Eitenmiller (2007).

**Moisture Analysis**

In order to allow results to be reported on a dry-weight basis, the moisture content of all samples was analyzed gravimetrically using the AACC 24-15A method.

**Data Calculation**

Folate content was determined using a standard curve. A separate curve was generated for each plate using folic acid in a pH 7 buffer. All standard curves fit a 4-point regression line with a $r^2$ value >0.99. The linear portion of the standard curve was calculated using Microsoft Excel (Microsoft Corp. Redmond, WA) and was used to determine the folic acid content of the samples. For each sample, the absorption values that fell within the linear portion of the standard curve were averaged. All statistical analyses were done using Statistical Analysis Software (SAS Institute, Cary, NC) using the ADX and proc mixed functions.

**Results and Discussion**

In the screening study using masa made from CMF, there was no significant decrease in folate in any treatment variable combination. Due to the fractional design

used, only main effects and two order interaction effects could be accurately determined. However, by using the hierarchical ordering principle described by Wu and Hamada (2000), the amount of information lost by fractionating the number of runs was small in comparison to the benefit of a reduced number of runs, which was ideal for a screening experiment. Although folate losses were observed in CMF treatment samples prepared in the laboratory, there was no statistical difference in folate retention between any combination of effects and no combination had greater than 18% loss. Therefore no attempt was made to augment the design with additional runs.

While data from the screening study did not help explain the large folate losses observed in the earlier commercial study of Dunn et al. (2008), it was in agreement with results from other studies evaluating the stability of folic acid in different food matrices. Nisha et al. (2005) heated a pure folic acid solution as well as fortified cowpeas to a temperature range of 50 – 120°C for up to 60min. They found no significant degradation of folic acid up to 80°C, which suggests folic acid in an aqueous solution and in food form are heat stable. de Brouwer et al. (2007) found folic acid to be 100% stable between a pH range of 2 to 10 even when heated to 100°C for two minutes or incubated at 37°C for two hours. In addition, Ball (2006) reports folic acid to be stable to both heat and alkaline pH. These results are consistent with the observation of no pH or temperature induced losses in folic acid in the current study.

It was expected that some formulation or process variables could be selected from the fractional-factorial design to narrow the focus during the commercial mill study. However, the results of this screening experiment indicated that pH, iron source, and

masa holding temperature and time had no effect on folate stability in CMF masa. Consequently, it was determined that some other mill-dependent factors were probably responsible for the degradation observed in prior studies. Samples were therefore pulled from different stages and times within the commercial process to monitor the folate loss.

Folate losses observed in masa samples prepared during the Guadalajara mill study were much more similar to results reported by Dunn (2008). While pre-bake masa holding time had no significant effect on folate retention in CMF masa samples in the laboratory, Table 3 shows that holding the hot masa before baking had a significant effect on the stability of folate in commercial masa prepared from nixtamal. Masa samples prepared with encapsulated folic acid went from 66% folate retention at 0.5hr to 40% retention at 4hr. Samples containing unencapsulated folic acid went from 51% retention at 0.5hr to 27% retention at 4hr. These results indicate a significant ($p<.0001$) difference in stability between the two premixes, with the encapsulated premix having, on average, a 14% higher retention over the 4-hour holding time. Of particular interest is the observation that there was no significant decrease in native folate throughout the entire transition from corn to nixtamal to masa to baked tortilla, as measured in the unfortified control samples (see Table 3). It appears that the native folate in the corn is protected from degradation, and the loss of folate in the fortified samples is probably coming exclusively at the expense of added folic acid. By contrast, de Brouwer et al. (2007) found that folic acid was stable across wide heat and pH ranges, but different forms of naturally occurring folate were not. They postulated that some natural forms of folate readily undergo interconversion to other products. However, their study was conducted

using pure solutions of folate and folic acid and not on folate in a food matrix. The location of folate in the corn matrix in this study possibly had a protective effect on the native folate. McKillop et al. (2002) concluded that the stability of folate depends highly on the food itself as well as the cooking method.

The significantly higher loss of folate in the commercial nixtamal mill, as compared to the screening study, confirms that factors specific to the commercial mill were causing the degradation. One important process variable present in the mill, but not in the laboratory was the grinding step. Masa in the Guadalajara study was prepared by passing fortified nixtamal through volcanic grinding stones. No similar process step was used in the lab study with CMF. The intense localized heat generated during the grinding process could result in a degradation of folate. The masa manufactured in the Guadalajara study came out of the grinding stones at 55° C and remained very near that temperature for the entire 4 hour holding time. Riaz et al. (2009) showed that up to 50% of folic acid can be degraded in an extrusion process at 80° C. Leskova et al. (2006) found that folic acid in various food matrices can be lost due to decomposition during extended heating. Holding the masa at 55° C for up to four hours could have contributed to the loss of folic acid.

In addition to the high heat generated by the mill stones, the volcanic grinding stones are typically high in iron and other metals. It is possible that oxidative reactions could be taking place on the surface of the hot stones, leading to the degradation of folate. On the other hand, Burton et al. (2008) also passed fortified nixtamal through volcanic grinding stones and showed much less folate degradation than observed in the present

study. However, masa holding time in the Burton study was less than 30 minutes. Kariluoto et al. (2006-1) found moderate losses of folic acid (26-28%), similar to the results of Burton et al., during extrusion of rye. They concluded that high-temperature-short-time processes result in much lower folate losses than high-temperature-long-time processes—such as those existing during storage of hot masa in the mill. The time-dependent nature of folate degradation in Table 3 indicates that post-grind holding losses are significant.

The significant loss of folate during extended holding of masa prior to baking could be caused by the accelerated chemical reaction rate at high temperatures. However, another possible explanation is that much of the folate loss is coming from bacterial degradation. Considerable fermentation of the masa had taken place by the time the 4-hour samples were collected. The masa was still acceptable, but had a distinct fermented smell. Kariluoto et al. (2006-2) have shown that naturally present lactic-acid bacteria can cause significant folic acid loss in bread dough. A preliminary evaluation of the lactic-acid bacteria count in fresh and stored masa from Guadalajara indicates that the number of bacteria increased dramatically during storage. It is possible that the added folic acid was degraded by lactic-acid or other bacteria during fermentation of the freshly ground masa. It is well known that many strains of bacteria are unable to consume the native poly- $\gamma$ -glutamate forms of folate (Quinlivan et al., 2006) which would help explain the observation that naturally occurring folates were not degraded in the control samples.

In addition to folate losses in masa, Table 3 also compares the stability of folate in unbaked masa to that of baked tortillas held for up to 12 hours. No further loss of folate

was observed in the baked tortillas, even after storage for 12 hours at ambient temperature. The lack of further folate degradation in the baked tortillas lends support to the idea that microorganisms are causing much of the folate loss, since the oven bakes the tortillas at 290° C, which would significantly reduce the microbial population. These results also agree well with other studies that show the stability of folate through the baking process. Osseyi et al. (2001) found only a ~20% loss of added folic acid through a 25 minute baking time of wheat bread. Gujska and Majewska (2005) found a loss of 12 – 21% of added folic acid, but found no loss of endogenous folate, during baking for 30 – 40 minutes. Keagy et al. (1975) found only a ~11% loss of total folate from baking bread dough for 25min at 425° F. Burton et al. (2008) and Dunn et al. (2008) also found no loss of total folate during tortilla baking. The baking time of the tortillas in Guadalajara is <1min, strengthening the idea that high-temperature, short-time processes result in good retention of folates.

While the current study does indicate a significant loss of folate in the commercially fortified tortillas, there is still a significant increase in folate content in the finished product, and this increase is due to addition of folic acid. Chun et al. (2006) used dietary folate equivalents (DFE) to more realistically show the benefit of fortifying with folic acid. The use of DFE to report activity is based on folic acid being 1.7 times more bioavailable then naturally occurring folates. Thus µg DFE can be calculated using the following formula: µg DFE = µg food folate + (1.7 x µg folic acid). For our purposes, we are interested in the gain of DFE's from the control tortillas to the fortified tortillas. Using an average concentration of 0.26µg folate / gram tortilla (dwb) in the

control tortillas, we found that the tortillas made from the premix containing unencapsulated folic acid had on average 9.3 times more DFE's then the control. The tortillas fortified with encapsulated folic acid had on average 12.5 times more DFE's then the control.

As previously mentioned, using encapsulated folic acid in the premix led to a 14% increase in folate stability. Using premix with lipid-encapsulated folic acid has been estimated to increase the price of the finished tortilla by 0.004 pesos per kilogram or about 0.04% of the total price, as compared to the use of unencapsulated folic acid (personal communication, Steve Schorn). This seems reasonable for a 14% increase in folic acid content, especially considering the recent change in recommendations on folic acid intake to 400-800 DFE's/day for women of child-bearing age (USPSTF, 2009). An increase in 14% folic acid could make meeting that recommendation significantly easier.

**Conclusions**

Fortifying masa flour with folic acid seems to be a viable method to increase the folate intake of those who regularly consume flour-derived products. Folate in masa flour was not affected by iron fortification, heat, or pH within the ranges evaluated. The commercial nixtamal mill study confirmed, however, the results of Dunn et al. (2008), indicating that significant folate loss occurs during the traditional manufacturing process. This study has shown that folate stability in nixtamal tortillas can be increased by minimizing the time of holding the masa prior to baking and by using encapsulated folic acid in the premix. Future research could focus on developing other methods to improve

the stability of this important vitamin, including the possibility of adding acidulants or other preservatives to limit the potential loss of folic acid caused by microbial growth.

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Table I

$2^2 \times 2^{3-1}$ Split Plot Fractional Factorial Design Used in the Fortified Corn Masa Flour Screening Study

| Run | Iron Source[a] | Iron Level (mg/kg)[b] | Temperature (°C) | Time (min)[c] | pH |
|---|---|---|---|---|---|
| 1 | E | 40 | 25 | 30 | 9 |
| 2 | E | 40 | 25 | 120 | 5 |
| 3 | E | 40 | 35 | 30 | 5 |
| 4 | E | 40 | 35 | 120 | 9 |
| 5 | F | 40 | 25 | 30 | 5 |
| 6 | F | 40 | 25 | 120 | 9 |
| 7 | F | 40 | 35 | 30 | 9 |
| 8 | F | 40 | 35 | 120 | 5 |
| 9 | E | 80 | 25 | 30 | 5 |
| 10 | E | 80 | 25 | 120 | 9 |
| 11 | E | 80 | 35 | 30 | 9 |
| 12 | E | 80 | 35 | 120 | 5 |
| 13 | F | 80 | 25 | 30 | 9 |
| 14 | F | 80 | 25 | 120 | 5 |
| 15 | F | 80 | 35 | 30 | 5 |
| 16 | F | 80 | 35 | 120 | 9 |

[a] E = Electrolytic Iron F = Ferrous Fumarate

[b] The levels indicate mg iron per kg masa flour

[c] Masa holding time prior to analysis

Table II

Composition of Micronutrient Premix Used in the Fortified Corn Masa Flour Screening Study

| Micronutrient | Commercial form | Concentration g/kg[a] |
|---|---|---|
| Vitamin B1 | Thiamin mononitrate | 3.55 |
| Vitamin B2 | Riboflavin | 1.73 |
| Niacin | Nicotinamide | 20.22 |
| Folic acid | Folic acid | 1.29 |
| Zinc | Zinc oxide | 29.05 |

[a] Concentration in premix (remainder = starch carrier and flow agent)

Table III

Folic Acid Content (ug/100g ± SD) of Dried Corn, Nixtamal, and Tortilla Samples Collected from the Commercial Mill in Guadalajara[a]

| Samples | Control | Unencapsulated | Encapsulated |
|---|---|---|---|
| Corn | 0.34a | | |
| Nixtamal | 0.28a | | |
| Masa .5 hr | 0.24aA | 1.81aB | 2.43aC |
| Masa 1 hr | 0.26aA | 1.58abB | 2.08abC |
| Masa 2 hr | 0.25aA | 1.39bB | 1.98bC |
| Masa 4 hr | 0.23aA | 0.98cB | 1.44cC |
| SE[b] | 0.098 | 0.098 | 0.102 |
| | | | |
| Prebake Masa | 0.27aA | 1.60aB | 1.90aC |
| Tortilla 0 hr | 0.28aA | 1.53aB | 2.05aC |
| Tortilla 12hr a[c] | 0.20aA | 1.45aB | 1.94aC |
| Tortilla 12hr r[d] | 0.25aA | 1.61aB | 2.06aC |
| SE[b] | 0.050 | 0.050 | 0.052 |

[a] Like lower-case letters within columns and capital letters within rows indicate no significant difference ($p>0.05$)

[b] SE, standard error of the mean

[c] a=uncontrolled ambient storage (22-26°C)

[d] r=refrigerated storage (5°C)

# APPENDICES

# APPENDIX A

# VITAMIN ANALYSIS DATA FROM THE GUADALAJARA STUDY

Contained in this appendix is the raw data for all the samples analyzed. Each page contains the analysis of one plate containing up to five samples. The top table on all the pages contains all the final information including the final concentration of folic acid in the sample. The key to interpret the sample I.D.'s is below:

- First Letter signifies the first letter of the product (C=Corn, N=Nixtamal, M=Masa, T=Tortilla, P=premix)
- Second designation is the treatment (Ctrl=control, F1=Encapsulated, F2=Unencapsulated)
- Third designation is the day
- Fourth designation is the time of storage (a=ambient, f=fridge for tortilla samples)
- Fifth designation is which of the duplicate samples (a or b) taken in Guadalajara.

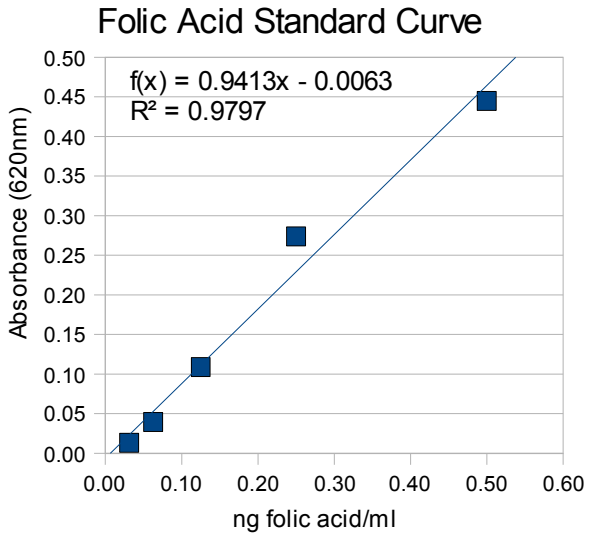
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ng/ml) (dwb) | Theoretical folic acid conc (ng/ml) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-24 Plate 1 | M-Ctrl-D1-0-a | 0.9908 | 2 | 0.450 | 0.0450 | 2.5 | 0.1135 | 55.79 | 0.257 | n/a | n/a |
| | M-Ctrl-D1-.5-a | 1.0284 | | 0.429 | 0.0429 | 2.5 | 0.1043 | 54.29 | 0.228 | n/a | n/a |
| | M-Ctrl-D1-.5-b | 1.0525 | | 0.469 | 0.0469 | 2.5 | 0.1115 | 54.72 | 0.246 | n/a | n/a |
| | M-Ctrl-D1-0-b | 1.0244 | | 0.542 | 0.0542 | 2.5 | 0.1322 | 55.48 | 0.297 | n/a | n/a |
| | M-F2-D1-0-a | 1.0824 | | 4.157 | 0.4157 | 2.5 | 0.9602 | 54.42 | 2.107 | 3.546 | 59.41 |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.017 | 0.010 | -0.005 | -0.004 | 0.011 | 0.006 | 0.005 | 0.009 | 0.008 | 0.014 | 0.049 | 0.068 |
| B | 0.038 | 0.041 | -0.001 | 0.006 | 0.003 | 0.014 | 0.009 | 0.009 | 0.020 | 0.026 | 0.091 | 0.123 |
| C | 0.105 | 0.113 | 0.013 | 0.023 | 0.021 | 0.016 | 0.021 | 0.024 | 0.030 | 0.023 | 0.232 | 0.280 |
| D | 0.283 | 0.265 | 0.038 | 0.045 | 0.040 | 0.042 | 0.053 | 0.038 | 0.061 | 0.059 | 0.421 | 0.487 |
| E | 0.450 | 0.440 | 0.095 | 0.102 | 0.086 | 0.089 | 0.103 | 0.112 | 0.120 | 0.119 | 0.584 | 0.610 |
| F | 0.635 | 0.621 | 0.221 | 0.225 | 0.218 | 0.215 | 0.227 | 0.218 | 0.246 | 0.267 | 0.711 | 0.769 |
| G | 0.737 | 0.785 | 0.462 | 0.466 | 0.436 | 0.402 | 0.406 | 0.426 | 0.460 | 0.459 | 0.869 | 0.891 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | M-Ctrl-D1-0-a | M-Ctrl-D1-.5-a | M-Ctrl-D1-.5-b | M-Ctrl-D1-0-b | M-F2-D1-0-a |
|---|---|---|---|---|---|---|
| A | 0.013 | -0.005 | 0.009 | 0.007 | 0.011 | 0.059 |
| B | 0.040 | 0.002 | 0.009 | 0.009 | 0.023 | 0.107 |
| C | 0.109 | 0.018 | 0.019 | 0.023 | 0.026 | 0.256 |
| D | 0.274 | 0.041 | 0.041 | 0.046 | 0.060 | 0.454 |
| E | 0.445 | 0.098 | 0.088 | 0.107 | 0.119 | 0.597 |
| F | 0.628 | 0.223 | 0.217 | 0.222 | 0.256 | 0.740 |
| G | 0.761 | 0.464 | 0.419 | 0.416 | 0.460 | 0.880 |




| M-Ctrl-D1-0-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | -0.005 | 0.0016 | 64 | 0.1054 | | |
| B | 0.002 | 0.0093 | 32 | 0.2975 | | |
| C | 0.018 | 0.0259 | 16 | 0.4139 | | |
| D | 0.041 | 0.0505 | 8 | 0.4041 | Average | 0.450 |
| E | 0.098 | 0.1110 | 4 | 0.4439 | STDEV | 0.043 |
| F | 0.223 | 0.2436 | 2 | 0.4872 | %RSD | 9.50 |
| G | 0.464 | 0.4997 | 1 | 0.4997 | Horrat | 0.45 |

| M-Ctrl-D1-.5-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.009 | 0.0157 | 64 | 1.0063 | | |
| B | 0.009 | 0.0159 | 32 | 0.5082 | | |
| C | 0.019 | 0.0263 | 16 | 0.4215 | | |
| D | 0.041 | 0.0500 | 8 | 0.3999 | Average | 0.429 |
| E | 0.088 | 0.0998 | 4 | 0.3990 | STDEV | 0.038 |
| F | 0.217 | 0.2370 | 2 | 0.4740 | %RSD | 8.75 |
| G | 0.419 | 0.4517 | 1 | 0.4517 | Horrat | 0.41 |

| M-Ctrl-D1-0-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.011 | 0.0180 | 64 | 1.1524 | | |
| B | 0.023 | 0.0310 | 32 | 0.9927 | | |
| C | 0.026 | 0.0347 | 16 | 0.5550 | | |
| D | 0.060 | 0.0708 | 8 | 0.5660 | Average | 0.542 |
| E | 0.119 | 0.1334 | 4 | 0.5337 | STDEV | 0.029 |
| F | 0.256 | 0.2790 | 2 | 0.5581 | %RSD | 5.30 |
| G | 0.460 | 0.4948 | 1 | 0.4948 | Horrat | 0.25 |

| M-Ctrl-D1-.5-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.007 | 0.0140 | 64 | 0.8975 | | |
| B | 0.009 | 0.0159 | 32 | 0.5099 | | |
| C | 0.023 | 0.0306 | 16 | 0.4895 | | |
| D | 0.046 | 0.0551 | 8 | 0.4411 | Average | 0.469 |
| E | 0.107 | 0.1204 | 4 | 0.4815 | STDEV | 0.020 |
| F | 0.222 | 0.2430 | 2 | 0.4859 | %RSD | 4.36 |
| G | 0.416 | 0.4485 | 1 | 0.4485 | Horrat | 0.20 |

| M-F2-D1-0-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.059 | 0.0691 | 64 | 4.423 | | |
| B | 0.107 | 0.1200 | 32 | 3.841 | | |
| C | 0.256 | 0.2784 | 16 | 4.455 | | |
| D | 0.454 | 0.4886 | 8 | 3.909 | Average | 4.157 |
| E | 0.597 | 0.6408 | 4 | 2.563 | STDEV | 0.327 |
| F | 0.740 | 0.7928 | 2 | 1.586 | %RSD | 7.86 |
| G | 0.880 | 0.9417 | 1 | 0.942 | Horrat | 0.37 |

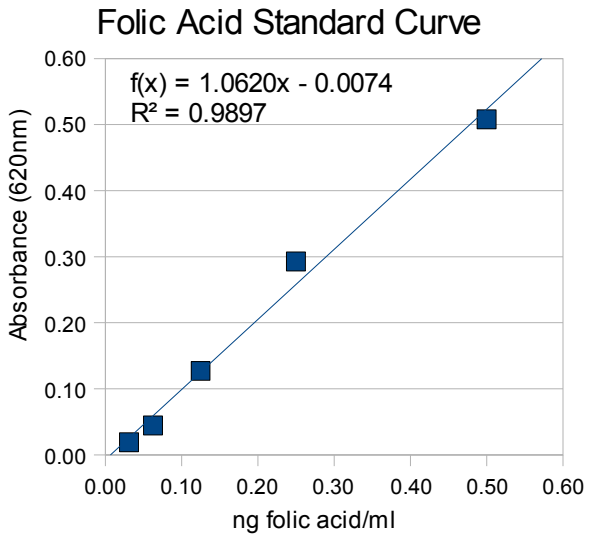
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ng/ml) (dwb) | Theoretical folic acid conc (ng/ml) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-24 Plate 2 | M-F2-D1-.5-a | 1.0277 | 2 | 3.930 | 0.3930 | 2.5 | 0.9560 | 53.93 | 2.075 | 3.511 | 59.1 |
| | M-F2-D1-0-b | 1.0356 | | 3.256 | 0.3256 | 2.5 | 0.7860 | 54.87 | 1.742 | 3.579 | 48.66 |
| | M-F1-D1-0-b | 0.9971 | | 4.638 | 0.4638 | 2.5 | 1.1629 | 52.99 | 2.474 | 3.518 | 70.32 |
| | M-F2-D1-.5-b | 1.0126 | | 3.768 | 0.3768 | 2.5 | 0.9303 | 53.32 | 1.993 | 3.468 | 57.47 |
| | M-F1-D1-0-a | 1.0635 | | 4.659 | 0.4659 | 2.5 | 1.0952 | 53.36 | 2.348 | 3.546 | 66.22 |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.024 | 0.014 | 0.045 | 0.040 | 0.049 | 0.040 | 0.061 | 0.068 | 0.053 | 0.049 | 0.057 | 0.069 |
| B | 0.047 | 0.042 | 0.118 | 0.136 | 0.105 | 0.093 | 0.143 | 0.154 | 0.116 | 0.124 | 0.152 | 0.140 |
| C | 0.123 | 0.131 | 0.273 | 0.263 | 0.219 | 0.193 | 0.363 | 0.314 | 0.245 | 0.271 | 0.322 | 0.353 |
| D | 0.315 | 0.271 | 0.456 | 0.481 | 0.437 | 0.437 | 0.534 | 0.511 | 0.472 | 0.434 | 0.530 | 0.557 |
| E | 0.521 | 0.495 | 0.674 | 0.691 | 0.552 | 0.620 | 0.703 | 0.727 | 0.611 | 0.679 | 0.737 | 0.730 |
| F | 0.666 | 0.681 | 0.817 | 0.827 | 0.822 | 0.787 | 0.806 | 0.852 | 0.780 | 0.780 | 0.830 | 0.867 |
| G | 0.760 | 0.785 | 0.922 | 0.928 | 0.911 | 0.930 | 0.936 | 0.934 | 0.903 | 0.892 | 0.959 | 0.950 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | M-F2-D1-.5-a | M-F2-D1-0-b | M-F1-D1-0-b | M-F2-D1-.5-b | M-F1-D1-0-a |
|---|---|---|---|---|---|---|
| A | 0.019 | 0.043 | 0.045 | 0.065 | 0.051 | 0.063 |
| B | 0.045 | 0.127 | 0.099 | 0.149 | 0.120 | 0.146 |
| C | 0.127 | 0.268 | 0.206 | 0.339 | 0.258 | 0.338 |
| D | 0.293 | 0.468 | 0.437 | 0.523 | 0.453 | 0.544 |
| E | 0.508 | 0.682 | 0.586 | 0.715 | 0.645 | 0.734 |
| F | 0.673 | 0.822 | 0.805 | 0.829 | 0.780 | 0.848 |
| G | 0.772 | 0.925 | 0.921 | 0.935 | 0.897 | 0.955 |




| M-F2-D1-.5-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.043 | 0.0472 | 64 | 3.023 | | |
| B | 0.127 | 0.1266 | 32 | 4.052 | | |
| C | 0.268 | 0.2596 | 16 | 4.153 | | |
| D | 0.468 | 0.4481 | 8 | 3.585 | Average | 3.930 |
| E | 0.682 | 0.6496 | 4 | 2.599 | STDEV | 0.303 |
| F | 0.822 | 0.7811 | 2 | 1.562 | %RSD | 7.71 |
| G | 0.925 | 0.8779 | 1 | 0.878 | Horrat | 0.36 |

| M-F2-D1-0-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.045 | 0.0491 | 64 | 3.144 | | |
| B | 0.099 | 0.1000 | 32 | 3.200 | | |
| C | 0.206 | 0.2010 | 16 | 3.216 | | |
| D | 0.437 | 0.4189 | 8 | 3.351 | Average | 3.256 |
| E | 0.586 | 0.5587 | 4 | 2.235 | STDEV | 0.083 |
| F | 0.805 | 0.7647 | 2 | 1.529 | %RSD | 2.55 |
| G | 0.921 | 0.8741 | 1 | 0.874 | Horrat | 0.12 |

| M-F2-D1-.5-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.051 | 0.0549 | 64 | 3.511 | | |
| B | 0.120 | 0.1201 | 32 | 3.842 | | |
| C | 0.258 | 0.2496 | 16 | 3.994 | | |
| D | 0.453 | 0.4335 | 8 | 3.468 | Average | 3.768 |
| E | 0.645 | 0.6147 | 4 | 2.459 | STDEV | 0.271 |
| F | 0.780 | 0.7412 | 2 | 1.482 | %RSD | 7.19 |
| G | 0.897 | 0.8520 | 1 | 0.852 | Horrat | 0.34 |

| M-F1-D1-0-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.065 | 0.0678 | 64 | 4.337 | | |
| B | 0.149 | 0.1470 | 32 | 4.704 | | |
| C | 0.339 | 0.3260 | 16 | 5.215 | | |
| D | 0.523 | 0.4994 | 8 | 3.995 | Average | 4.638 |
| E | 0.715 | 0.6805 | 4 | 2.722 | STDEV | 0.613 |
| F | 0.829 | 0.7879 | 2 | 1.576 | %RSD | 13.21 |
| G | 0.935 | 0.8875 | 1 | 0.888 | Horrat | 0.62 |

| M-F1-D1-0-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.063 | 0.0664 | 64 | 4.253 | | |
| B | 0.146 | 0.1446 | 32 | 4.626 | | |
| C | 0.338 | 0.3250 | 16 | 5.200 | | |
| D | 0.544 | 0.5189 | 8 | 4.151 | Average | 4.659 |
| E | 0.734 | 0.6978 | 4 | 2.791 | STDEV | 0.525 |
| F | 0.848 | 0.8059 | 2 | 1.612 | %RSD | 11.28 |
| G | 0.955 | 0.9060 | 1 | 0.906 | Horrat | 0.53 |

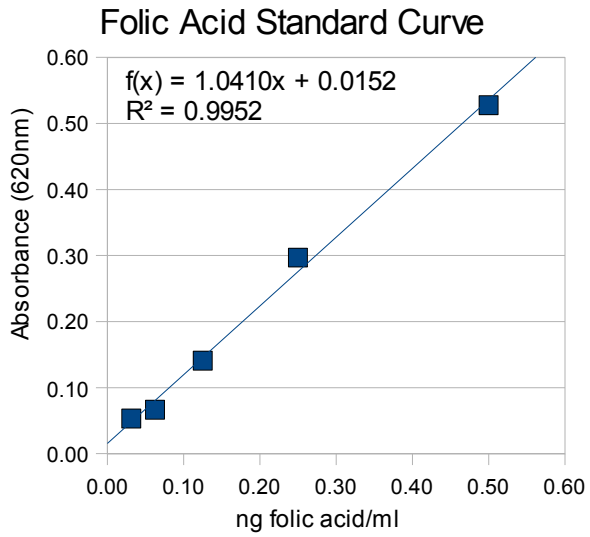
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ng/ml) (dwb) | Theoretical folic acid conc (ng/ml) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-24 Plate 3 | M-F1-D1-.5-a | 1.0613 | 2 | 5.443 | 0.544 | 2.5 | 1.2820 | 54.33 | 2.807 | 3.614 | 77.68 |
| | M-F1-D1-.5-b | 1.0425 | | 5.488 | 0.549 | 2.5 | 1.3160 | 58.54 | 3.174 | 3.955 | 80.26 |
| | M-Ctrl-D1-4-a | 1.0155 | | 0.683 | 0.068 | 1.67 | 0.1121 | 56.14 | 0.255 | n/a | n/a |
| | M-F1-D1-4-a | 1.0188 | | 6.587 | 0.659 | 1.67 | 1.0776 | 53.38 | 2.311 | 3.545 | 65.2 |
| | M-F2-D1-4-a | 1.0049 | | 3.212 | 0.321 | 1.67 | 0.5327 | 54.52 | 1.171 | 3.546 | 33.03 |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.063 | 0.043 | 0.108 | 0.114 | 0.115 | 0.140 | 0.033 | 0.089 | 0.115 | 0.122 | 0.078 | 0.069 |
| B | 0.070 | 0.063 | 0.178 | 0.205 | 0.202 | 0.209 | 0.029 | 0.046 | 0.226 | 0.224 | 0.107 | 0.108 |
| C | 0.149 | 0.132 | 0.360 | 0.386 | 0.388 | 0.383 | 0.045 | 0.087 | 0.472 | 0.465 | 0.237 | 0.253 |
| D | 0.298 | 0.296 | 0.634 | 0.695 | 0.652 | 0.660 | 0.070 | 0.116 | 0.686 | 0.669 | 0.425 | 0.456 |
| E | 0.545 | 0.510 | 0.776 | 0.744 | 0.835 | 0.762 | 0.175 | 0.206 | 0.862 | 0.868 | 0.706 | 0.735 |
| F | 0.736 | 0.745 | 0.942 | 0.934 | 0.932 | 0.947 | 0.379 | 0.352 | 0.988 | 0.987 | 0.896 | 0.900 |
| G | 0.857 | 0.888 | 1.050 | 1.014 | 1.011 | 1.051 | 0.689 | 0.629 | 1.041 | 1.037 | 1.018 | 1.019 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | M-F1-D1-.5-a | M-F1-D1-.5-b | M-Ctrl-D1-4-a | M-F1-D1-4-a | M-F2-D1-4-a |
|---|---|---|---|---|---|---|
| A | 0.053 | 0.111 | 0.127 | 0.061 | 0.119 | 0.073 |
| B | 0.066 | 0.192 | 0.205 | 0.038 | 0.225 | 0.107 |
| C | 0.141 | 0.373 | 0.385 | 0.066 | 0.468 | 0.245 |
| D | 0.297 | 0.665 | 0.656 | 0.093 | 0.678 | 0.441 |
| E | 0.528 | 0.760 | 0.798 | 0.191 | 0.865 | 0.721 |
| F | 0.740 | 0.938 | 0.939 | 0.366 | 0.987 | 0.898 |
| G | 0.872 | 1.032 | 1.031 | 0.659 | 1.039 | 1.018 |




| M-F1-D1-.5-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.111 | 0.0915 | 64 | 5.859 | | |
| B | 0.192 | 0.1694 | 32 | 5.421 | | |
| C | 0.373 | 0.3438 | 16 | 5.500 | | |
| D | 0.665 | 0.6237 | 8 | 4.990 | Average | 5.443 |
| E | 0.760 | 0.7152 | 4 | 2.861 | STDEV | 0.357 |
| F | 0.938 | 0.8862 | 2 | 1.772 | %RSD | 6.56 |
| G | 1.032 | 0.9769 | 1 | 0.977 | Horrat | 0.31 |

| M-F1-D1-.5-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.127 | 0.1077 | 64 | 6.892 | | |
| B | 0.205 | 0.1827 | 32 | 5.847 | | |
| C | 0.385 | 0.3557 | 16 | 5.691 | | |
| D | 0.656 | 0.6157 | 8 | 4.926 | Average | 5.488 |
| E | 0.798 | 0.7522 | 4 | 3.009 | STDEV | 0.493 |
| F | 0.939 | 0.8877 | 2 | 1.775 | %RSD | 8.98 |
| G | 1.031 | 0.9757 | 1 | 0.976 | Horrat | 0.42 |

| M-F1-D1-4-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.119 | 0.0992 | 64 | 6.351 | | |
| B | 0.225 | 0.2015 | 32 | 6.449 | | |
| C | 0.468 | 0.4351 | 16 | 6.961 | | |
| D | 0.678 | 0.6365 | 8 | 5.092 | Average | 6.587 |
| E | 0.865 | 0.8163 | 4 | 3.265 | STDEV | 0.966 |
| F | 0.987 | 0.9340 | 2 | 1.868 | %RSD | 14.66 |
| G | 1.039 | 0.9838 | 1 | 0.984 | Horrat | 0.69 |

| M-Ctrl-D1-4-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.061 | 0.0439 | 64 | 2.810 | | |
| B | 0.038 | 0.0214 | 32 | 0.686 | | |
| C | 0.066 | 0.0488 | 16 | 0.781 | | |
| D | 0.093 | 0.0749 | 8 | 0.599 | Average | 0.683 |
| E | 0.191 | 0.1687 | 4 | 0.675 | STDEV | 0.065 |
| F | 0.366 | 0.3367 | 2 | 0.673 | %RSD | 9.49 |
| G | 0.659 | 0.6183 | 1 | 0.618 | Horrat | 0.44 |

| M-F2-D1-4-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.073 | 0.0558 | 64 | 3.572 | | |
| B | 0.107 | 0.0886 | 32 | 2.836 | | |
| C | 0.245 | 0.2207 | 16 | 3.531 | | |
| D | 0.441 | 0.4086 | 8 | 3.269 | Average | 3.212 |
| E | 0.721 | 0.6776 | 4 | 2.710 | STDEV | 0.351 |
| F | 0.898 | 0.8482 | 2 | 1.696 | %RSD | 10.93 |
| G | 1.018 | 0.9635 | 1 | 0.963 | Horrat | 0.51 |

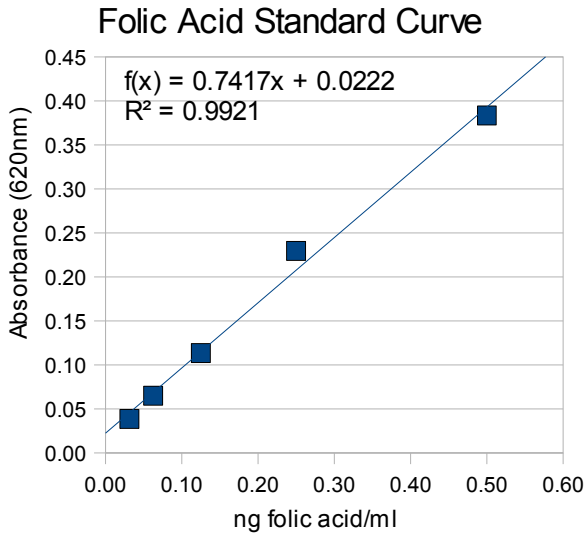
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-25 Plate 1 | M-F2-D2-0-a | 1.0091 | 2 | 2.186 | 0.2186 | 2.5 | 0.5417 | 53.47 | 1.164 | 3.544 | 32.85 |
| | M-F2-D2-.5-a | 1.0013 | | 3.437 | 0.3437 | 2.5 | 0.8581 | 52.95 | 1.824 | 3.508 | 52 |
| | M-Ctrl-D2-0-a | 1.0181 | | 0.463 | 0.0463 | 2.5 | 0.1138 | 55.81 | 0.257 | n/a | n/a |
| | M-Ctrl-D2-.5-a | 1.0336 | | 0.453 | 0.0453 | 2.5 | 0.1095 | 53.83 | 0.237 | n/a | n/a |
| | | | | | | | | | | | |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.040 | 0.037 | 0.041 | 0.114 | 0.055 | 0.059 | 0.029 | 0.042 | 0.024 | 0.031 | 0.031 | 0.032 |
| B | 0.063 | 0.067 | 0.071 | 0.261 | 0.099 | 0.114 | 0.034 | 0.046 | 0.040 | 0.041 | 0.046 | 0.045 |
| C | 0.113 | 0.114 | 0.125 | 0.126 | 0.156 | 0.215 | 0.043 | 0.056 | 0.038 | 0.073 | 0.082 | 0.084 |
| D | 0.226 | 0.233 | 0.233 | 0.268 | 0.337 | 0.290 | 0.056 | 0.075 | 0.056 | 0.070 | 0.235 | 0.186 |
| E | 0.412 | 0.355 | 0.367 | 0.374 | 0.405 | 0.456 | 0.107 | 0.121 | 0.108 | 0.111 | 0.453 | 0.444 |
| F | 0.511 | 0.485 | 0.535 | 0.484 | 0.567 | 0.537 | 0.200 | 0.197 | 0.201 | 0.184 | 0.672 | 0.614 |
| G | 0.613 | 0.580 | 0.621 | 0.631 | 0.628 | 0.648 | 0.343 | 0.318 | 0.367 | 0.333 | 0.817 | 0.822 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | M-F2-D2-0-a | M-F2-D2-.5-a | M-Ctrl-D2-0-a | M-Ctrl-D2-.5-a | M-Ctrl-D2-0-b |
|---|---|---|---|---|---|---|
| A | 0.038 | 0.078 | 0.057 | 0.036 | 0.028 | 0.031 |
| B | 0.065 | 0.166 | 0.107 | 0.040 | 0.040 | 0.046 |
| C | 0.113 | 0.125 | 0.185 | 0.050 | 0.055 | 0.083 |
| D | 0.229 | 0.250 | 0.314 | 0.066 | 0.063 | 0.210 |
| E | 0.383 | 0.370 | 0.430 | 0.114 | 0.109 | 0.448 |
| F | 0.498 | 0.509 | 0.552 | 0.199 | 0.193 | 0.643 |
| G | 0.597 | 0.626 | 0.638 | 0.330 | 0.350 | 0.819 |




| M-F2-D2-0-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.078 | 0.0749 | 64 | 4.7923 | | |
| B | 0.166 | 0.1937 | 32 | 6.1993 | | |
| C | 0.125 | 0.1389 | 16 | 2.2217 | | |
| D | 0.250 | 0.3076 | 8 | 2.4607 | Average | 2.186 |
| E | 0.370 | 0.4692 | 4 | 1.8770 | STDEV | 0.293 |
| F | 0.509 | 0.6569 | 2 | 1.3137 | %RSD | 13.42 |
| G | 0.626 | 0.8138 | 1 | 0.8138 | Horrat | 0.63 |

| M-F2-D2-.5-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.057 | 0.0470 | 64 | 3.0061 | | |
| B | 0.107 | 0.1140 | 32 | 3.6495 | | |
| C | 0.185 | 0.2197 | 16 | 3.5160 | | |
| D | 0.314 | 0.3932 | 8 | 3.1456 | Average | 3.437 |
| E | 0.430 | 0.5503 | 4 | 2.2014 | STDEV | 0.261 |
| F | 0.552 | 0.7139 | 2 | 1.4278 | %RSD | 7.60 |
| G | 0.638 | 0.8306 | 1 | 0.8306 | Horrat | 0.36 |

| M-Ctrl-D2-.5-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.028 | 0.0073 | 64 | 0.4649 | | |
| B | 0.040 | 0.0243 | 32 | 0.7782 | | |
| C | 0.055 | 0.0447 | 16 | 0.7149 | | |
| D | 0.063 | 0.0549 | 8 | 0.4394 | Average | 0.453 |
| E | 0.109 | 0.1175 | 4 | 0.4699 | STDEV | 0.015 |
| F | 0.193 | 0.2302 | 2 | 0.4604 | %RSD | 3.25 |
| G | 0.350 | 0.4419 | 1 | 0.4419 | Horrat | 0.15 |

| M-Ctrl-D2-0-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.036 | 0.0183 | 64 | 1.1682 | | |
| B | 0.040 | 0.0239 | 32 | 0.7653 | | |
| C | 0.050 | 0.0373 | 16 | 0.5962 | | |
| D | 0.066 | 0.0584 | 8 | 0.4674 | Average | 0.463 |
| E | 0.114 | 0.1237 | 4 | 0.4947 | STDEV | 0.034 |
| F | 0.199 | 0.2378 | 2 | 0.4756 | %RSD | 7.32 |
| G | 0.330 | 0.4154 | 1 | 0.4154 | Horrat | 0.34 |

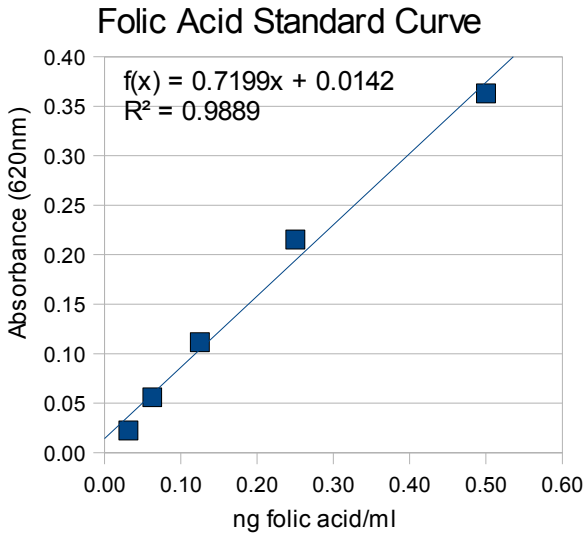
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-25 Plate 2 | M-F2-D2-0-b | 1.0133 | 2 | 2.489 | 0.2489 | 2.5 | 0.6140 | 53.38 | 1.317 | 3.538 | 37.23 |
| | M-F1-D2-.5-a | 1.0121 | | 3.312 | 0.3312 | 2.5 | 0.8182 | 55.12 | 1.823 | 3.499 | 52.11 |
| | M-F1-D2-.5-b | 1.0123 | | 3.519 | 0.3519 | 2.5 | 0.8691 | 54.89 | 1.927 | 3.482 | 55.33 |
| | M-F2-D1-4-b | 1.0279 | | 3.286 | 0.3286 | 1.67 | 0.5339 | 54.48 | 1.173 | 3.550 | 33.04 |
| | M-F1-D2-0-a | 1.0070 | | 2.767 | 0.2767 | 2.5 | 0.6869 | 55.58 | 1.546 | 3.532 | 43.78 |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.023 | 0.022 | 0.026 | 0.036 | 0.050 | 0.052 | 0.067 | 0.067 | 0.064 | 0.066 | 0.054 | 0.051 |
| B | 0.055 | 0.057 | 0.074 | 0.084 | 0.124 | 0.117 | 0.123 | 0.145 | 0.122 | 0.125 | 0.091 | 0.090 |
| C | 0.106 | 0.117 | 0.136 | 0.132 | 0.205 | 0.213 | 0.221 | 0.244 | 0.229 | 0.203 | 0.187 | 0.189 |
| D | 0.203 | 0.228 | 0.216 | 0.240 | 0.348 | 0.330 | 0.341 | 0.354 | 0.321 | 0.343 | 0.314 | 0.307 |
| E | 0.353 | 0.373 | 0.352 | 0.409 | 0.448 | 0.498 | 0.494 | 0.548 | 0.470 | 0.479 | 0.449 | 0.502 |
| F | 0.462 | 0.476 | 0.506 | 0.502 | 0.589 | 0.557 | 0.568 | 0.641 | 0.586 | 0.562 | 0.562 | 0.534 |
| G | 0.574 | 0.591 | 0.651 | 0.591 | 0.694 | 0.668 | 0.676 | 0.656 | 0.712 | 0.682 | 0.686 | 0.664 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | M-F2-D2-0-b | M-F1-D2-.5-a | M-F1-D2-.5-b | M-F2-D1-4-b | M-F1-D2-0-a |
|---|---|---|---|---|---|---|
| A | 0.022 | 0.031 | 0.051 | 0.067 | 0.065 | 0.053 |
| B | 0.056 | 0.079 | 0.120 | 0.134 | 0.123 | 0.091 |
| C | 0.112 | 0.134 | 0.209 | 0.233 | 0.216 | 0.188 |
| D | 0.215 | 0.228 | 0.339 | 0.347 | 0.332 | 0.311 |
| E | 0.363 | 0.381 | 0.473 | 0.521 | 0.475 | 0.476 |
| F | 0.469 | 0.504 | 0.573 | 0.605 | 0.574 | 0.548 |
| G | 0.582 | 0.621 | 0.681 | 0.666 | 0.697 | 0.675 |




| M-F2-D2-0-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.031 | 0.0232 | 64 | 1.4849 | | |
| B | 0.079 | 0.0898 | 32 | 2.8739 | | |
| C | 0.134 | 0.1667 | 16 | 2.6671 | | |
| D | 0.228 | 0.2969 | 8 | 2.3754 | Average | 2.489 |
| E | 0.381 | 0.5095 | 4 | 2.0378 | STDEV | 0.363 |
| F | 0.504 | 0.6807 | 2 | 1.3613 | %RSD | 14.61 |
| G | 0.621 | 0.8431 | 1 | 0.8431 | Horrat | 0.68 |

| M-F1-D2-.5-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.051 | 0.0552 | 64 | 3.5298 | | |
| B | 0.120 | 0.1202 | 32 | 3.8455 | | |
| C | 0.209 | 0.2038 | 16 | 3.2614 | | |
| D | 0.339 | 0.3266 | 8 | 2.6126 | Average | 3.312 |
| E | 0.473 | 0.4524 | 4 | 1.8097 | STDEV | 0.524 |
| F | 0.573 | 0.5468 | 2 | 1.0936 | %RSD | 15.82 |
| G | 0.681 | 0.6482 | 1 | 0.6482 | Horrat | 0.74 |

| M-F2-D1-4-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.065 | 0.0684 | 64 | 4.3796 | | |
| B | 0.123 | 0.1231 | 32 | 3.9389 | | |
| C | 0.216 | 0.2101 | 16 | 3.3616 | | |
| D | 0.332 | 0.3198 | 8 | 2.5587 | Average | 3.286 |
| E | 0.475 | 0.4541 | 4 | 1.8163 | STDEV | 0.693 |
| F | 0.574 | 0.5472 | 2 | 1.0943 | %RSD | 21.09 |
| G | 0.697 | 0.6630 | 1 | 0.6630 | Horrat | 0.99 |

| M-F1-D2-.5-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.065 | 0.0684 | 64 | 4.3796 | | |
| B | 0.123 | 0.1231 | 32 | 3.9389 | | |
| C | 0.216 | 0.2101 | 16 | 3.3616 | | |
| D | 0.332 | 0.3198 | 8 | 2.5587 | Average | 3.286 |
| E | 0.475 | 0.4541 | 4 | 1.8163 | STDEV | 0.693 |
| F | 0.574 | 0.5472 | 2 | 1.0943 | %RSD | 21.09 |
| G | 0.697 | 0.6630 | 1 | 0.6630 | Horrat | 0.99 |

| M-F1-D2-0-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.053 | 0.0565 | 64 | 3.614 | | |
| B | 0.091 | 0.0925 | 32 | 2.961 | | |
| C | 0.188 | 0.1840 | 16 | 2.943 | | |
| D | 0.311 | 0.2996 | 8 | 2.396 | Average | 2.767 |
| E | 0.476 | 0.4548 | 4 | 1.819 | STDEV | 0.321 |
| F | 0.548 | 0.5230 | 2 | 1.046 | %RSD | 11.6 |
| G | 0.675 | 0.6423 | 1 | 0.642 | Horrat | 0.54 |

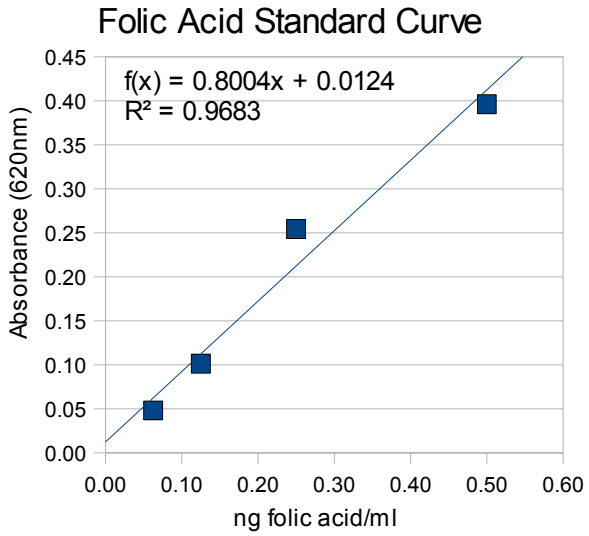
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-25 Plate 3 | M-Ctrl-D2-.5-b | 1.0189 | 2 | 0.499 | 0.0499 | 2.5 | 0.1224 | 53.24 | 0.262 | n/a | n/a |
| | M-F2-D2-.5-b | 1.0489 | | 2.680 | 0.2680 | 2.5 | 0.6387 | 52.96 | 1.358 | 3.508 | 38.7 |
| | M-F1-D2-0-b | 1.0207 | | 2.255 | 0.2255 | 2.5 | 0.5524 | 55.47 | 1.241 | 3.524 | 35.2 |
| | M-Ctrl-D1-4-b | 1.0831 | | 0.570 | 0.0570 | 1.67 | 0.0878 | 56.25 | 0.201 | n/a | n/a |
| | M-F1-D1-4-b | 1.0731 | | 5.466 | 0.5466 | 1.67 | 0.8490 | 54.2 | 1.854 | 3.604 | 51.43 |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | -0.077 | -0.084 | -0.071 | -0.069 | -0.065 | -0.069 | -0.063 | -0.067 | -0.054 | -0.038 | -0.049 | -0.027 |
| B | 0.042 | 0.054 | 0.029 | 0.012 | 0.097 | 0.093 | 0.084 | 0.085 | 0.044 | 0.039 | 0.191 | 0.202 |
| C | 0.099 | 0.103 | 0.072 | 0.023 | 0.214 | 0.205 | 0.188 | 0.158 | 0.060 | 0.063 | 0.368 | 0.360 |
| D | 0.254 | 0.255 | 0.057 | 0.058 | 0.343 | 0.361 | 0.304 | 0.301 | 0.090 | 0.096 | 0.521 | 0.515 |
| E | 0.417 | 0.375 | 0.102 | 0.124 | 0.506 | 0.530 | 0.464 | 0.462 | 0.168 | 0.179 | 0.696 | 0.648 |
| F | 0.546 | 0.561 | 0.229 | 0.221 | 0.675 | 0.701 | 0.661 | 0.569 | 0.330 | 0.335 | 0.774 | 0.751 |
| G | 0.653 | 0.701 | 0.432 | 0.408 | 0.761 | 0.780 | 0.734 | 0.752 | 0.506 | 0.497 | 0.834 | 0.854 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | M-Ctrl-D2-.5-b | M-F2-D2-.5-b | M-F1-D2-0-b | M-Ctrl-D1-4-b | M-F1-D1-4-b |
|---|---|---|---|---|---|---|
| A | -0.081 | -0.070 | -0.067 | -0.065 | -0.046 | -0.038 |
| B | 0.048 | 0.020 | 0.095 | 0.084 | 0.041 | 0.197 |
| C | 0.101 | 0.048 | 0.210 | 0.173 | 0.062 | 0.364 |
| D | 0.254 | 0.057 | 0.352 | 0.303 | 0.093 | 0.518 |
| E | 0.396 | 0.113 | 0.518 | 0.463 | 0.173 | 0.672 |
| F | 0.554 | 0.225 | 0.688 | 0.615 | 0.332 | 0.763 |
| G | 0.677 | 0.420 | 0.771 | 0.743 | 0.502 | 0.844 |




| M-Ctrl-D2-.5-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | -0.070 | -0.0817 | 64 | -5.2307 | | |
| B | 0.020 | 0.0100 | 32 | 0.3194 | | |
| C | 0.048 | 0.0445 | 16 | 0.7114 | | |
| D | 0.057 | 0.0563 | 8 | 0.4507 | Average | 0.499 |
| E | 0.113 | 0.1259 | 4 | 0.5037 | STDEV | 0.034 |
| F | 0.225 | 0.2660 | 2 | 0.5320 | %RSD | 6.89 |
| G | 0.420 | 0.5091 | 1 | 0.5091 | Horrat | 0.32 |

| M-F2-D2-.5-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | -0.067 | -0.0789 | 64 | -5.0524 | | |
| B | 0.095 | 0.0769 | 32 | 2.4613 | | |
| C | 0.210 | 0.1868 | 16 | 2.9890 | | |
| D | 0.352 | 0.3236 | 8 | 2.5888 | Average | 2.680 |
| E | 0.518 | 0.4831 | 4 | 1.9324 | STDEV | 0.275 |
| F | 0.688 | 0.6463 | 2 | 1.2925 | %RSD | 10.27 |
| G | 0.771 | 0.7258 | 1 | 0.7258 | Horrat | 0.48 |

| M-Ctrl-D1-4-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | -0.046 | -0.0587 | 64 | -3.7582 | | |
| B | 0.041 | 0.0252 | 32 | 0.8060 | | |
| C | 0.062 | 0.0447 | 16 | 0.7150 | | |
| D | 0.093 | 0.0748 | 8 | 0.5980 | Average | 0.570 |
| E | 0.173 | 0.1517 | 4 | 0.6068 | STDEV | 0.069 |
| F | 0.332 | 0.3047 | 2 | 0.6095 | %RSD | 12.08 |
| G | 0.502 | 0.4673 | 1 | 0.4673 | Horrat | 0.57 |

| M-F1-D2-0-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | -0.065 | -0.0772 | 64 | -4.9417 | | |
| B | 0.084 | 0.0665 | 32 | 2.1278 | | |
| C | 0.173 | 0.1517 | 16 | 2.4272 | | |
| D | 0.303 | 0.2764 | 8 | 2.2111 | Average | 2.255 |
| E | 0.463 | 0.4300 | 4 | 1.7200 | STDEV | 0.155 |
| F | 0.615 | 0.5762 | 2 | 1.1524 | %RSD | 6.85 |
| G | 0.743 | 0.6993 | 1 | 0.6993 | Horrat | 0.32 |

| M-F1-D1-4-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | -0.038 | -0.0514 | 64 | -3.291 | | |
| B | 0.197 | 0.1743 | 32 | 5.577 | | |
| C | 0.364 | 0.3347 | 16 | 5.356 | | |
| D | 0.518 | 0.4834 | 8 | 3.867 | Average | 5.466 |
| E | 0.672 | 0.6310 | 4 | 2.524 | STDEV | 0.156 |
| F | 0.763 | 0.7178 | 2 | 1.436 | %RSD | 2.86 |
| G | 0.844 | 0.7961 | 1 | 0.796 | Horrat | 0.13 |

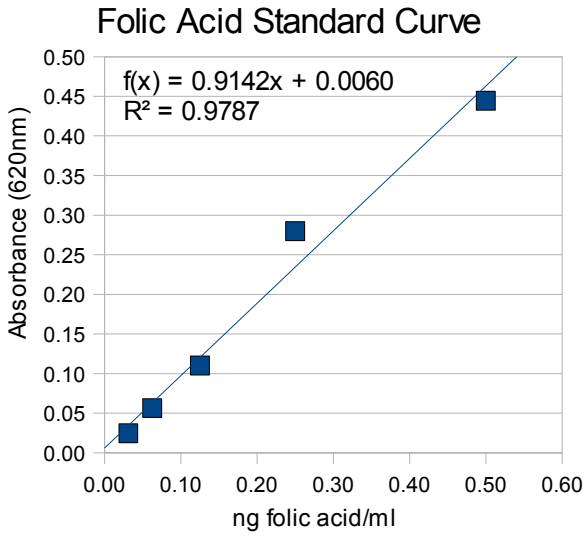
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-26 Plate 1 | M-F2-D2-4-a | 1.0340 | 2 | 2.110 | 0.2110 | 1.67 | 0.3401 | 55.21 | 0.759 | 3.672 | 20.68 |
| | M-F1-D2-4-a | 1.0061 | | 1.784 | 0.1784 | 2.5 | 0.4433 | 53.59 | 0.955 | 3.391 | 28.16 |
| | M-Ctrl-D2-4-a | 1.0194 | | 0.994 | 0.0994 | 1 | 0.0975 | 54.51 | 0.214 | n/a | n/a |
| | T-F2-D1-0-b | 1.0127 | | 3.791 | 0.3791 | 2.5 | 0.9359 | 45.48 | 1.717 | 3.517 | 48.81 |
| | T-F2-D2-0-b | 1.0047 | | 2.882 | 0.2882 | 2.5 | 0.7170 | 45.32 | 1.311 | 3.530 | 37.15 |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.030 | 0.019 | 0.024 | 0.023 | 0.024 | 0.033 | 0.019 | 0.023 | 0.057 | 0.076 | 0.042 | 0.049 |
| B | 0.058 | 0.055 | 0.060 | 0.063 | 0.055 | 0.060 | 0.035 | 0.032 | 0.114 | 0.119 | 0.083 | 0.079 |
| C | 0.115 | 0.105 | 0.133 | 0.131 | 0.108 | 0.103 | 0.062 | 0.058 | 0.250 | 0.253 | 0.170 | 0.187 |
| D | 0.278 | 0.281 | 0.264 | 0.286 | 0.217 | 0.248 | 0.119 | 0.121 | 0.355 | 0.392 | 0.338 | 0.363 |
| E | 0.464 | 0.425 | 0.472 | 0.429 | 0.389 | 0.359 | 0.244 | 0.253 | 0.495 | 0.563 | 0.557 | 0.535 |
| F | 0.559 | 0.566 | 0.594 | 0.532 | 0.526 | 0.509 | 0.455 | 0.438 | 0.630 | 0.670 | 0.666 | 0.549 |
| G | 0.657 | 0.667 | 0.721 | 0.662 | 0.634 | 0.587 | 0.639 | 0.606 | 0.770 | 0.754 | 0.743 | 0.773 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | M-F2-D2-4-a | M-F1-D2-4-a | M-Ctrl-D2-4-a | T-F2-D1-0-b | T-F2-D2-0-b |
|---|---|---|---|---|---|---|
| A | 0.025 | 0.024 | 0.029 | 0.021 | 0.066 | 0.046 |
| B | 0.056 | 0.061 | 0.058 | 0.034 | 0.116 | 0.081 |
| C | 0.110 | 0.132 | 0.105 | 0.060 | 0.251 | 0.178 |
| D | 0.280 | 0.275 | 0.232 | 0.120 | 0.374 | 0.351 |
| E | 0.444 | 0.451 | 0.374 | 0.248 | 0.529 | 0.546 |
| F | 0.562 | 0.563 | 0.517 | 0.447 | 0.650 | 0.608 |
| G | 0.662 | 0.692 | 0.610 | 0.623 | 0.762 | 0.758 |




| M-F2-D2-4-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.024 | 0.0194 | 64 | 1.2393 | | |
| B | 0.061 | 0.0603 | 32 | 1.9288 | | |
| C | 0.132 | 0.1380 | 16 | 2.2087 | | |
| D | 0.275 | 0.2945 | 8 | 2.3562 | Average | 2.110 |
| E | 0.451 | 0.4863 | 4 | 1.9451 | STDEV | 0.208 |
| F | 0.563 | 0.6094 | 2 | 1.2188 | %RSD | 9.88 |
| G | 0.692 | 0.7499 | 1 | 0.7499 | Horrat | 0.46 |

| M-F1-D2-4-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.029 | 0.0249 | 64 | 1.5963 | | |
| B | 0.058 | 0.0564 | 32 | 1.8045 | | |
| C | 0.105 | 0.1088 | 16 | 1.7406 | | |
| D | 0.232 | 0.2475 | 8 | 1.9803 | Average | 1.784 |
| E | 0.374 | 0.4027 | 4 | 1.6108 | STDEV | 0.154 |
| F | 0.517 | 0.5592 | 2 | 1.1185 | %RSD | 8.61 |
| G | 0.610 | 0.6612 | 1 | 0.6612 | Horrat | 0.40 |

| T-F2-D1-0-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.066 | 0.0660 | 64 | 4.2250 | | |
| B | 0.116 | 0.1207 | 32 | 3.8627 | | |
| C | 0.251 | 0.2684 | 16 | 4.2941 | | |
| D | 0.374 | 0.4020 | 8 | 3.2164 | Average | 3.791 |
| E | 0.529 | 0.5725 | 4 | 2.2899 | STDEV | 0.542 |
| F | 0.650 | 0.7047 | 2 | 1.4094 | %RSD | 14.31 |
| G | 0.762 | 0.8267 | 1 | 0.8267 | Horrat | 0.67 |

| M-Ctrl-D2-4-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.021 | 0.0166 | 64 | 1.0642 | | |
| B | 0.034 | 0.0302 | 32 | 0.9679 | | |
| C | 0.060 | 0.0592 | 16 | 0.9469 | | |
| D | 0.120 | 0.1249 | 8 | 0.9994 | Average | 0.994 |
| E | 0.248 | 0.2651 | 4 | 1.0604 | STDEV | 0.045 |
| F | 0.447 | 0.4822 | 2 | 0.9643 | %RSD | 4.51 |
| G | 0.623 | 0.6746 | 1 | 0.6746 | Horrat | 0.21 |

| T-F2-D2-0-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.046 | 0.0435 | 64 | 2.783 | | |
| B | 0.081 | 0.0817 | 32 | 2.615 | | |
| C | 0.178 | 0.1884 | 16 | 3.015 | | |
| D | 0.351 | 0.3769 | 8 | 3.015 | Average | 2.882 |
| E | 0.546 | 0.5910 | 4 | 2.364 | STDEV | 0.231 |
| F | 0.608 | 0.6580 | 2 | 1.316 | %RSD | 8.02 |
| G | 0.758 | 0.8225 | 1 | 0.822 | Horrat | 0.38 |

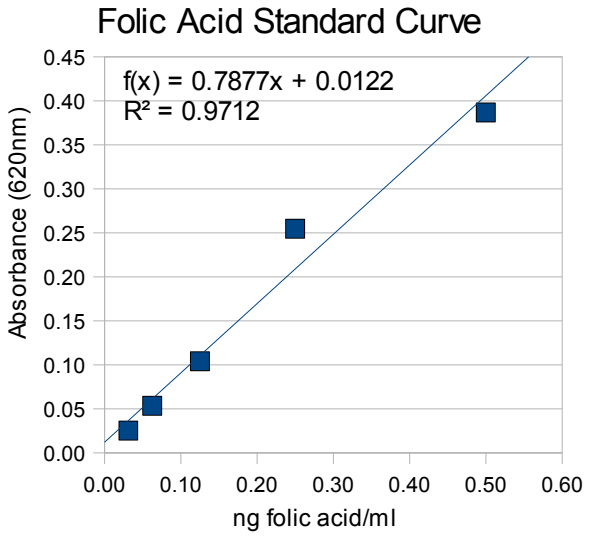
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-26 Plate 2 | T-F1-D1-0-b | 1.0328 | 2 | 5.386 | 0.5386 | 2.5 | 1.3038 | 44.18 | 2.336 | 3.612 | 64.66 |
| | T-F1-D2-0-b | 1.0152 | | 4.849 | 0.4849 | 2.5 | 1.1942 | 44.41 | 2.148 | 3.510 | 61.21 |
| | T-F2-D2-0-a | 1.0285 | | 3.360 | 0.3360 | 2.5 | 0.8168 | 43.41 | 1.443 | 3.530 | 40.89 |
| | T-Ctrl-D1-0-b | 1.0289 | | 1.715 | 0.1715 | 1 | 0.1667 | 43.29 | 0.294 | n/a | n/a |
| | T-Ctrl-D2-0-b | 1.0027 | | 1.684 | 0.1684 | 1 | 0.1679 | 43.16 | 0.295 | n/a | n/a |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.023 | 0.027 | 0.069 | 0.072 | 0.064 | 0.073 | 0.056 | 0.057 | 0.035 | 0.040 | 0.032 | 0.037 |
| B | 0.049 | 0.058 | 0.150 | 0.153 | 0.131 | 0.138 | 0.099 | 0.092 | 0.061 | 0.047 | 0.042 | 0.033 |
| C | 0.099 | 0.109 | 0.287 | 0.306 | 0.270 | 0.286 | 0.183 | 0.173 | 0.091 | 0.082 | 0.076 | 0.090 |
| D | 0.267 | 0.242 | 0.466 | 0.473 | 0.433 | 0.465 | 0.339 | 0.292 | 0.172 | 0.209 | 0.162 | 0.194 |
| E | 0.391 | 0.382 | 0.622 | 0.567 | 0.616 | 0.568 | 0.531 | 0.504 | 0.361 | 0.388 | 0.360 | 0.427 |
| F | 0.590 | 0.564 | 0.706 | 0.760 | 0.727 | 0.695 | 0.673 | 0.644 | 0.544 | 0.649 | 0.597 | 0.607 |
| G | 0.673 | 0.699 | 0.817 | 0.831 | 0.803 | 0.762 | 0.792 | 0.776 | 0.705 | 0.743 | 0.704 | 0.712 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | T-F1-D1-0-b | T-F1-D2-0-b | T-F2-D2-0-a | T-Ctrl-D1-0-b | T-Ctrl-D2-0-b |
|---|---|---|---|---|---|---|
| A | 0.025 | 0.070 | 0.068 | 0.056 | 0.037 | 0.034 |
| B | 0.053 | 0.152 | 0.135 | 0.096 | 0.054 | 0.037 |
| C | 0.104 | 0.297 | 0.278 | 0.178 | 0.087 | 0.083 |
| D | 0.254 | 0.470 | 0.449 | 0.315 | 0.191 | 0.178 |
| E | 0.387 | 0.595 | 0.592 | 0.517 | 0.374 | 0.393 |
| F | 0.577 | 0.733 | 0.711 | 0.658 | 0.596 | 0.602 |
| G | 0.686 | 0.824 | 0.782 | 0.784 | 0.724 | 0.708 |




| T-F1-D1-0-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.070 | 0.0736 | 64 | 4.7118 | | |
| B | 0.152 | 0.1771 | 32 | 5.6668 | | |
| C | 0.297 | 0.3612 | 16 | 5.7797 | | |
| D | 0.470 | 0.5810 | 8 | 4.6479 | Average | 5.386 |
| E | 0.595 | 0.7395 | 4 | 2.9579 | STDEV | 0.587 |
| F | 0.733 | 0.9151 | 2 | 1.8301 | %RSD | 10.89 |
| G | 0.824 | 1.0308 | 1 | 1.0308 | Horrat | 0.51 |

| T-F1-D2-0-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.068 | 0.0715 | 64 | 4.5777 | | |
| B | 0.135 | 0.1556 | 32 | 4.9782 | | |
| C | 0.278 | 0.3377 | 16 | 5.4039 | | |
| D | 0.449 | 0.5547 | 8 | 4.4376 | Average | 4.849 |
| E | 0.592 | 0.7364 | 4 | 2.9457 | STDEV | 0.435 |
| F | 0.711 | 0.8869 | 2 | 1.7738 | %RSD | 8.97 |
| G | 0.782 | 0.9779 | 1 | 0.9779 | Horrat | 0.42 |

| T-Ctrl-D1-0-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.037 | 0.0319 | 64 | 2.0387 | | |
| B | 0.054 | 0.0531 | 32 | 1.6978 | | |
| C | 0.087 | 0.0944 | 16 | 1.5111 | | |
| D | 0.191 | 0.2265 | 8 | 1.8118 | Average | 1.715 |
| E | 0.374 | 0.4598 | 4 | 1.8392 | STDEV | 0.149 |
| F | 0.596 | 0.7414 | 2 | 1.4828 | %RSD | 8.69 |
| G | 0.724 | 0.9031 | 1 | 0.9031 | Horrat | 0.41 |

| T-F2-D2-0-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.056 | 0.0562 | 64 | 3.5987 | | |
| B | 0.096 | 0.1062 | 32 | 3.3999 | | |
| C | 0.178 | 0.2102 | 16 | 3.3635 | | |
| D | 0.315 | 0.3849 | 8 | 3.0793 | Average | 3.360 |
| E | 0.517 | 0.6415 | 4 | 2.5659 | STDEV | 0.214 |
| F | 0.658 | 0.8205 | 2 | 1.6411 | %RSD | 6.37 |
| G | 0.784 | 0.9794 | 1 | 0.9794 | Horrat | 0.30 |

| T-Ctrl-D2-0-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.034 | 0.0282 | 64 | 1.803 | | |
| B | 0.037 | 0.0320 | 32 | 1.023 | | |
| C | 0.083 | 0.0894 | 16 | 1.431 | | |
| D | 0.178 | 0.2107 | 8 | 1.685 | Average | 1.684 |
| E | 0.393 | 0.4837 | 4 | 1.935 | STDEV | 0.252 |
| F | 0.602 | 0.7489 | 2 | 1.498 | %RSD | 14.96 |
| G | 0.708 | 0.8834 | 1 | 0.883 | Horrat | 0.7 |

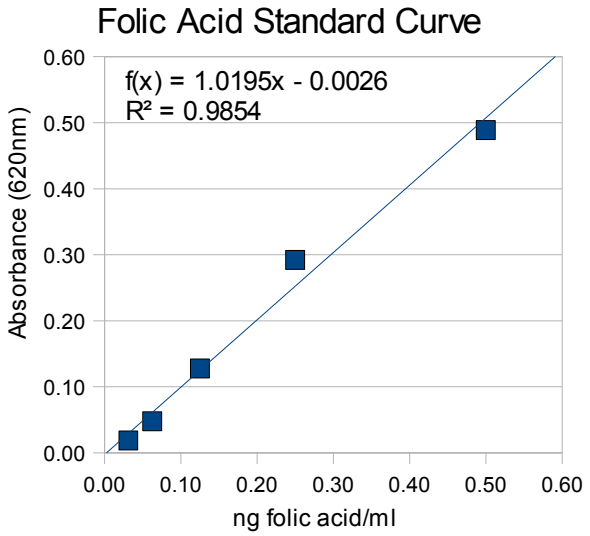
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-26 Plate 3 | T-F1-D2-0-a | 1.0276 | 2 | 3.846 | 0.3846 | 2.5 | 0.9356 | 44.39 | 1.682 | 3.612 | 46.58 |
| | T-F1-D1-0-a | 1.0024 | | 4.493 | 0.4493 | 2.5 | 1.1205 | 44.59 | 2.022 | 3.510 | 57.62 |
| | T-F2-D1-0-a | 1.0436 | | 3.901 | 0.3901 | 2.5 | 0.9345 | 42.85 | 1.635 | 3.530 | 46.32 |
| | T-Ctrl-D2-0-a | 1.0282 | | 1.512 | 0.1512 | 1 | 0.1471 | 44.93 | 0.267 | n/a | n/a |
| | T-Ctrl-D1-0-a | 1.0193 | | 1.597 | 0.1597 | 1 | 0.1567 | 44.04 | 0.280 | n/a | n/a |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.018 | 0.019 | 0.053 | 0.051 | 0.066 | 0.074 | 0.055 | 0.062 | 0.025 | 0.027 | 0.026 | 0.030 |
| B | 0.047 | 0.049 | 0.116 | 0.129 | 0.150 | 0.154 | 0.140 | 0.133 | 0.048 | 0.047 | 0.048 | 0.043 |
| C | 0.126 | 0.129 | 0.277 | 0.238 | 0.290 | 0.337 | 0.276 | 0.280 | 0.099 | 0.099 | 0.111 | 0.110 |
| D | 0.288 | 0.297 | 0.509 | 0.460 | 0.549 | 0.520 | 0.431 | 0.488 | 0.211 | 0.211 | 0.247 | 0.215 |
| E | 0.488 | 0.490 | 0.643 | 0.628 | 0.662 | 0.628 | 0.609 | 0.634 | 0.387 | 0.399 | 0.380 | 0.426 |
| F | 0.602 | 0.580 | 0.767 | 0.735 | 0.781 | 0.774 | 0.736 | 0.747 | 0.604 | 0.583 | 0.641 | 0.633 |
| G | 0.687 | 0.715 | 0.851 | 0.816 | 0.870 | 0.874 | 0.839 | 0.804 | 0.755 | 0.731 | 0.745 | 0.759 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | T-F1-D2-0-a | T-F1-D1-0-a | T-F2-D1-0-a | T-Ctrl-D2-0-a | T-Ctrl-D1-0-a |
|---|---|---|---|---|---|---|
| A | 0.018 | 0.052 | 0.070 | 0.058 | 0.026 | 0.028 |
| B | 0.048 | 0.123 | 0.152 | 0.137 | 0.047 | 0.045 |
| C | 0.128 | 0.257 | 0.313 | 0.278 | 0.099 | 0.111 |
| D | 0.292 | 0.485 | 0.534 | 0.460 | 0.211 | 0.231 |
| E | 0.489 | 0.636 | 0.645 | 0.621 | 0.393 | 0.403 |
| F | 0.591 | 0.751 | 0.778 | 0.742 | 0.593 | 0.637 |
| G | 0.701 | 0.833 | 0.872 | 0.821 | 0.743 | 0.752 |




| T-F1-D2-0-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.052 | 0.0483 | 64 | 3.0932 | | |
| B | 0.123 | 0.1176 | 32 | 3.7631 | | |
| C | 0.257 | 0.2496 | 16 | 3.9937 | | |
| D | 0.485 | 0.4725 | 8 | 3.7804 | Average | 3.846 |
| E | 0.636 | 0.6205 | 4 | 2.4822 | STDEV | 0.128 |
| F | 0.751 | 0.7336 | 2 | 1.4673 | %RSD | 3.34 |
| G | 0.833 | 0.8146 | 1 | 0.8146 | Horrat | 0.16 |

| T-F1-D1-0-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.070 | 0.0664 | 64 | 4.2477 | | |
| B | 0.152 | 0.1462 | 32 | 4.6776 | | |
| C | 0.313 | 0.3048 | 16 | 4.8761 | | |
| D | 0.534 | 0.5213 | 8 | 4.1702 | Average | 4.493 |
| E | 0.645 | 0.6299 | 4 | 2.5194 | STDEV | 0.339 |
| F | 0.778 | 0.7598 | 2 | 1.5195 | %RSD | 7.55 |
| G | 0.872 | 0.8526 | 1 | 0.8526 | Horrat | 0.35 |

| T-Ctrl-D2-0-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.026 | 0.0231 | 64 | 1.4775 | | |
| B | 0.047 | 0.0439 | 32 | 1.4054 | | |
| C | 0.099 | 0.0946 | 16 | 1.5137 | | |
| D | 0.211 | 0.2042 | 8 | 1.6337 | Average | 1.512 |
| E | 0.393 | 0.3827 | 4 | 1.5310 | STDEV | 0.083 |
| F | 0.593 | 0.5790 | 2 | 1.1579 | %RSD | 5.50 |
| G | 0.743 | 0.7259 | 1 | 0.7259 | Horrat | 0.26 |

| T-F2-D1-0-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.058 | 0.0546 | 64 | 3.4917 | | |
| B | 0.137 | 0.1316 | 32 | 4.2117 | | |
| C | 0.278 | 0.2697 | 16 | 4.3145 | | |
| D | 0.460 | 0.4483 | 8 | 3.5867 | Average | 3.901 |
| E | 0.621 | 0.6064 | 4 | 2.4257 | STDEV | 0.422 |
| F | 0.742 | 0.7246 | 2 | 1.4492 | %RSD | 10.81 |
| G | 0.821 | 0.8028 | 1 | 0.8028 | Horrat | 0.51 |

| T-Ctrl-D1-0-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.028 | 0.0248 | 64 | 1.587 | | |
| B | 0.045 | 0.0419 | 32 | 1.341 | | |
| C | 0.111 | 0.1060 | 16 | 1.696 | | |
| D | 0.231 | 0.2239 | 8 | 1.791 | Average | 1.597 |
| E | 0.403 | 0.3927 | 4 | 1.571 | STDEV | 0.169 |
| F | 0.637 | 0.6219 | 2 | 1.244 | %RSD | 10.56 |
| G | 0.752 | 0.7347 | 1 | 0.735 | Horrat | 0.49 |

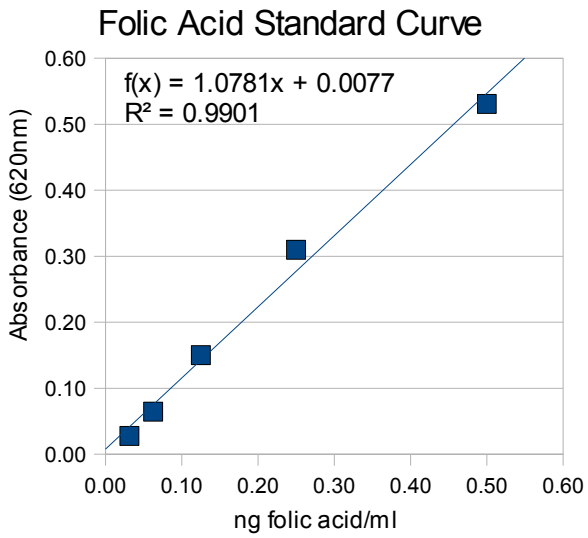
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-2 Plate 1 | M-F1-D1-2-a | 1.0095 | 2 | 5.157 | 0.5157 | 2.5 | 1.2772 | 54.14 | 2.785 | 3.600 | 77.37 |
| | M-F2-D2-4-b | 1.0158 | | 2.291 | 0.2291 | 1.67 | 0.3767 | 53.84 | 0.816 | 3.570 | 22.86 |
| | M-F1-D2-4-b | 1.0188 | | 1.682 | 0.1682 | 1.67 | 0.2758 | 55.53 | 0.620 | 3.529 | 17.58 |
| | M-F1-D1-2-b | 1.0065 | | 5.242 | 0.5242 | 2.5 | 1.3022 | 53.99 | 2.830 | 3.589 | 78.86 |
| | M-Ctrl-D2-4-b | 1.0070 | | 0.623 | 0.0623 | 1.67 | 0.1033 | 55.17 | 0.230 | n/a | n/a |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.028 | 0.027 | 0.078 | 0.084 | 0.042 | 0.041 | 0.036 | 0.040 | 0.088 | 0.091 | 0.016 | 0.031 |
| B | 0.063 | 0.066 | 0.204 | 0.193 | 0.087 | 0.083 | 0.065 | 0.069 | 0.186 | 0.198 | 0.035 | 0.032 |
| C | 0.180 | 0.120 | 0.357 | 0.392 | 0.204 | 0.152 | 0.131 | 0.122 | 0.373 | 0.371 | 0.052 | 0.047 |
| D | 0.322 | 0.298 | 0.604 | 0.602 | 0.322 | 0.323 | 0.208 | 0.210 | 0.587 | 0.570 | 0.089 | 0.087 |
| E | 0.560 | 0.501 | 0.754 | 0.761 | 0.534 | 0.541 | 0.428 | 0.447 | 0.757 | 0.775 | 0.179 | 0.177 |
| F | 0.706 | 0.732 | 0.884 | 0.884 | 0.735 | 0.774 | 0.641 | 0.654 | 0.917 | 0.876 | 0.387 | 0.321 |
| G | 0.818 | 0.844 | 1.006 | 0.981 | 0.897 | 0.888 | 0.835 | 0.820 | 0.983 | 0.955 | 0.627 | 0.609 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | M-F1-D1-2-a | M-F2-D2-4-b | M-F1-D2-4-b | M-F1-D1-2-b | M-Ctrl-D2-4-b |
|---|---|---|---|---|---|---|
| A | 0.028 | 0.081 | 0.042 | 0.038 | 0.089 | 0.024 |
| B | 0.065 | 0.199 | 0.085 | 0.067 | 0.192 | 0.034 |
| C | 0.150 | 0.375 | 0.178 | 0.127 | 0.372 | 0.049 |
| D | 0.310 | 0.603 | 0.323 | 0.209 | 0.578 | 0.088 |
| E | 0.531 | 0.758 | 0.538 | 0.437 | 0.766 | 0.178 |
| F | 0.719 | 0.884 | 0.754 | 0.648 | 0.897 | 0.354 |
| G | 0.831 | 0.993 | 0.893 | 0.828 | 0.969 | 0.618 |




M-F1-D1-2-a

| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
|---|---|---|---|---|---|---|
| A | 0.081 | 0.0680 | 64 | 4.3546 | | |
| B | 0.199 | 0.1772 | 32 | 5.6697 | | |
| C | 0.375 | 0.3405 | 16 | 5.4478 | | |
| D | 0.603 | 0.5521 | 8 | 4.4171 | Average | 5.157 |
| E | 0.758 | 0.6956 | 4 | 2.7826 | STDEV | 0.704 |
| F | 0.884 | 0.8130 | 2 | 1.6260 | %RSD | 13.65 |
| G | 0.993 | 0.9143 | 1 | 0.9143 | Horrat | 0.64 |

M-F2-D2-4-b

| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
|---|---|---|---|---|---|---|
| A | 0.042 | 0.0314 | 64 | 2.0125 | | |
| B | 0.085 | 0.0715 | 32 | 2.2871 | | |
| C | 0.178 | 0.1580 | 16 | 2.5284 | | |
| D | 0.323 | 0.2922 | 8 | 2.3376 | Average | 2.291 |
| E | 0.538 | 0.4916 | 4 | 1.9664 | STDEV | 0.213 |
| F | 0.754 | 0.6927 | 2 | 1.3853 | %RSD | 9.30 |
| G | 0.893 | 0.8208 | 1 | 0.8208 | Horrat | 0.44 |

M-F1-D2-4-b

| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
|---|---|---|---|---|---|---|
| A | 0.038 | 0.0280 | 64 | 1.7928 | | |
| B | 0.067 | 0.0551 | 32 | 1.7647 | | |
| C | 0.127 | 0.1104 | 16 | 1.7662 | | |
| D | 0.209 | 0.1868 | 8 | 1.4942 | Average | 1.682 |
| E | 0.437 | 0.3986 | 4 | 1.5942 | STDEV | 0.131 |
| F | 0.648 | 0.5938 | 2 | 1.1877 | %RSD | 7.82 |
| G | 0.828 | 0.7606 | 1 | 0.7606 | Horrat | 0.37 |

M-F1-D1-2-b

| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
|---|---|---|---|---|---|---|
| A | 0.089 | 0.0758 | 64 | 4.8533 | | |
| B | 0.192 | 0.1707 | 32 | 5.4634 | | |
| C | 0.372 | 0.3382 | 16 | 5.4107 | | |
| D | 0.578 | 0.5293 | 8 | 4.2341 | Average | 5.242 |
| E | 0.766 | 0.7034 | 4 | 2.8137 | STDEV | 0.338 |
| F | 0.897 | 0.8245 | 2 | 1.6491 | %RSD | 6.45 |
| G | 0.969 | 0.8916 | 1 | 0.8916 | Horrat | 0.30 |

M-Ctrl-D2-4-b

| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
|---|---|---|---|---|---|---|
| A | 0.024 | 0.0147 | 64 | 0.938 | | |
| B | 0.034 | 0.0243 | 32 | 0.776 | | |
| C | 0.049 | 0.0387 | 16 | 0.620 | | |
| D | 0.088 | 0.0746 | 8 | 0.597 | Average | 0.623 |
| E | 0.178 | 0.1582 | 4 | 0.633 | STDEV | 0.020 |
| F | 0.354 | 0.3211 | 2 | 0.642 | %RSD | 3.16 |
| G | 0.618 | 0.5660 | 1 | 0.566 | Horrat | 0.15 |

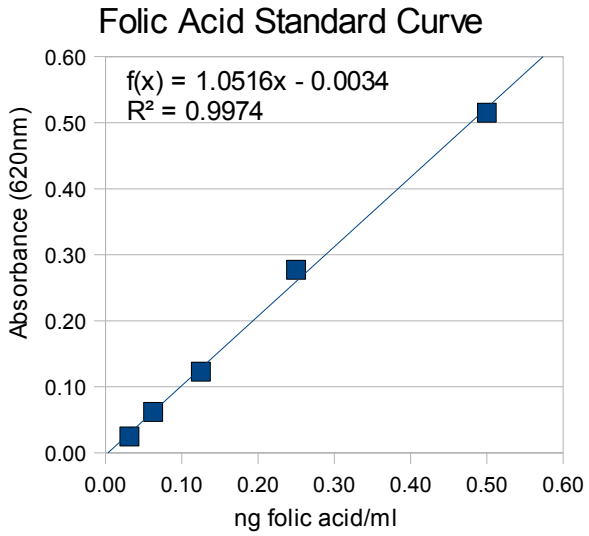
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-2 Plate 2 | M-F1-D1-1-a | 1.0231 | 2 | 5.402 | 0.5402 | 2.5 | 1.3199 | 53.98 | 2.868 | 3.588 | 79.93 |
| | M-F1-D1-1-b | 1.0091 | | 6.808 | 0.6808 | 2.5 | 1.6866 | 54.2 | 3.683 | 3.604 | 102.18 |
| | M-F2-D1-2-b | 1.0059 | | 3.962 | 0.3962 | 2.5 | 0.9848 | 53.79 | 2.131 | 3.501 | 60.87 |
| | M-F2-D1-1-a | 1.0668 | | 4.319 | 0.4319 | 2.5 | 1.0122 | 53.49 | 2.176 | 3.480 | 62.53 |
| | M-F2-D1-1-b | 1.0439 | | 4.086 | 0.4086 | 2.5 | 0.9786 | 53.75 | 2.116 | 3.498 | 60.49 |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.025 | 0.025 | 0.083 | 0.079 | 0.109 | 0.114 | 0.068 | 0.062 | 0.065 | 0.068 | 0.062 | 0.063 |
| B | 0.066 | 0.058 | 0.181 | 0.169 | 0.222 | 0.234 | 0.123 | 0.122 | 0.142 | 0.148 | 0.121 | 0.133 |
| C | 0.130 | 0.116 | 0.340 | 0.394 | 0.389 | 0.448 | 0.271 | 0.267 | 0.338 | 0.275 | 0.284 | 0.299 |
| D | 0.284 | 0.270 | 0.572 | 0.666 | 0.683 | 0.675 | 0.495 | 0.474 | 0.505 | 0.491 | 0.512 | 0.503 |
| E | 0.529 | 0.502 | 0.821 | 0.798 | 0.824 | 0.766 | 0.729 | 0.697 | 0.734 | 0.710 | 0.742 | 0.729 |
| F | 0.757 | 0.731 | 0.918 | 0.903 | 0.941 | 0.938 | 0.870 | 0.869 | 0.869 | 0.875 | 0.874 | 0.888 |
| G | 0.779 | 0.840 | 0.999 | 0.984 | 0.971 | 1.007 | 0.954 | 0.955 | 0.961 | 0.954 | 0.959 | 0.969 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | M-F1-D1-1-a | M-F1-D1-1-b | M-F2-D1-2-b | M-F2-D1-1-a | M-F2-D1-1-b |
|---|---|---|---|---|---|---|
| A | 0.025 | 0.081 | 0.111 | 0.065 | 0.066 | 0.062 |
| B | 0.062 | 0.175 | 0.228 | 0.122 | 0.145 | 0.127 |
| C | 0.123 | 0.367 | 0.418 | 0.269 | 0.306 | 0.291 |
| D | 0.277 | 0.619 | 0.679 | 0.484 | 0.498 | 0.508 |
| E | 0.515 | 0.809 | 0.795 | 0.713 | 0.722 | 0.736 |
| F | 0.744 | 0.911 | 0.940 | 0.869 | 0.872 | 0.881 |
| G | 0.809 | 0.991 | 0.989 | 0.955 | 0.957 | 0.964 |




| M-F1-D1-1-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.081 | 0.0803 | 64 | 5.1369 | | |
| B | 0.175 | 0.1699 | 32 | 5.4354 | | |
| C | 0.367 | 0.3520 | 16 | 5.6325 | | |
| D | 0.619 | 0.5912 | 8 | 4.7296 | Average | 5.402 |
| E | 0.809 | 0.7723 | 4 | 3.0893 | STDEV | 0.250 |
| F | 0.911 | 0.8689 | 2 | 1.7378 | %RSD | 4.62 |
| G | 0.991 | 0.9457 | 1 | 0.9457 | Horrat | 0.22 |

| M-F1-D1-1-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.111 | 0.1089 | 64 | 6.9711 | | |
| B | 0.228 | 0.2201 | 32 | 7.0430 | | |
| C | 0.418 | 0.4006 | 16 | 6.4097 | | |
| D | 0.679 | 0.6483 | 8 | 5.1866 | Average | 6.808 |
| E | 0.795 | 0.7589 | 4 | 3.0357 | STDEV | 0.347 |
| F | 0.940 | 0.8963 | 2 | 1.7927 | %RSD | 5.09 |
| G | 0.989 | 0.9437 | 1 | 0.9437 | Horrat | 0.24 |

| M-F2-D1-2-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.065 | 0.0652 | 64 | 4.1757 | | |
| B | 0.122 | 0.1195 | 32 | 3.8247 | | |
| C | 0.269 | 0.2588 | 16 | 4.1405 | | |
| D | 0.484 | 0.4636 | 8 | 3.7086 | Average | 3.962 |
| E | 0.713 | 0.6810 | 4 | 2.7239 | STDEV | 0.231 |
| F | 0.869 | 0.8297 | 2 | 1.6594 | %RSD | 5.84 |
| G | 0.955 | 0.9107 | 1 | 0.9107 | Horrat | 0.27 |

| M-F2-D1-1-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.066 | 0.0664 | 64 | 4.2487 | | |
| B | 0.145 | 0.1409 | 32 | 4.5091 | | |
| C | 0.306 | 0.2942 | 16 | 4.7078 | | |
| D | 0.498 | 0.4764 | 8 | 3.8113 | Average | 4.319 |
| E | 0.722 | 0.6899 | 4 | 2.7596 | STDEV | 0.387 |
| F | 0.872 | 0.8322 | 2 | 1.6643 | %RSD | 8.97 |
| G | 0.957 | 0.9132 | 1 | 0.9132 | Horrat | 0.42 |

| M-F2-D1-1-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.062 | 0.0625 | 64 | 4.002 | | |
| B | 0.127 | 0.1242 | 32 | 3.974 | | |
| C | 0.291 | 0.2802 | 16 | 4.483 | | |
| D | 0.508 | 0.4857 | 8 | 3.886 | Average | 4.086 |
| E | 0.736 | 0.7027 | 4 | 2.811 | STDEV | 0.269 |
| F | 0.881 | 0.8406 | 2 | 1.681 | %RSD | 6.59 |
| G | 0.964 | 0.9196 | 1 | 0.920 | Horrat | 0.31 |

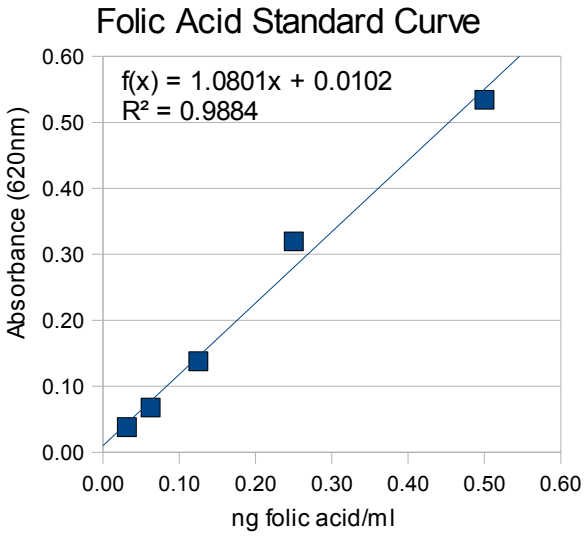
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-2 Plate 3 | M-Ctrl-D1-1-b | 1.0051 | 2 | 1.082 | 0.1082 | 1 | 0.1077 | 56.79 | 0.249 | n/a | n/a |
| | M-Ctrl-D1-2-a | 1.0716 | | 1.219 | 0.1219 | 1 | 0.1137 | 54.95 | 0.252 | n/a | n/a |
| | M-F2-D1-2-a | 1.0466 | | 3.885 | 0.3885 | 2.5 | 0.9279 | 53.52 | 1.996 | 3.501 | 57.02 |
| | M-Ctrl-D1-2-b | 1.0281 | | 1.348 | 0.1348 | 1 | 0.1312 | 55.17 | 0.293 | n/a | n/a |
| | M-Ctrl-D1-1-a | 1.0561 | | 1.215 | 0.1215 | 1 | 0.1150 | 56.76 | 0.266 | n/a | n/a |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.033 | 0.043 | 0.026 | 0.026 | 0.030 | 0.029 | 0.075 | 0.068 | 0.034 | 0.036 | 0.034 | 0.036 |
| B | 0.065 | 0.070 | 0.037 | 0.040 | 0.044 | 0.052 | 0.128 | 0.132 | 0.048 | 0.050 | 0.045 | 0.041 |
| C | 0.135 | 0.140 | 0.068 | 0.078 | 0.080 | 0.089 | 0.300 | 0.302 | 0.098 | 0.088 | 0.084 | 0.082 |
| D | 0.340 | 0.299 | 0.180 | 0.170 | 0.177 | 0.202 | 0.539 | 0.579 | 0.175 | 0.213 | 0.169 | 0.162 |
| E | 0.491 | 0.577 | 0.394 | 0.353 | 0.380 | 0.395 | 0.764 | 0.769 | 0.430 | 0.415 | 0.385 | 0.390 |
| F | 0.746 | 0.759 | 0.649 | 0.657 | 0.629 | 0.689 | 0.908 | 0.882 | 0.722 | 0.648 | 0.682 | 0.611 |
| G | 0.867 | 0.912 | 0.857 | 0.852 | 0.870 | 0.869 | 0.994 | 0.999 | 0.843 | 0.872 | 0.868 | 0.856 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | M-Ctrl-D1-1-b | M-Ctrl-D1-2-a | M-F2-D1-2-a | M-Ctrl-D1-2-b | M-Ctrl-D1-1-a |
|---|---|---|---|---|---|---|
| A | 0.038 | 0.026 | 0.030 | 0.071 | 0.035 | 0.035 |
| B | 0.068 | 0.038 | 0.048 | 0.130 | 0.049 | 0.043 |
| C | 0.138 | 0.073 | 0.084 | 0.301 | 0.093 | 0.083 |
| D | 0.320 | 0.175 | 0.189 | 0.559 | 0.194 | 0.166 |
| E | 0.534 | 0.373 | 0.387 | 0.766 | 0.423 | 0.388 |
| F | 0.752 | 0.653 | 0.659 | 0.895 | 0.685 | 0.646 |
| G | 0.889 | 0.854 | 0.870 | 0.996 | 0.858 | 0.862 |




| M-Ctrl-D1-1-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.026 | 0.0150 | 64 | 0.9595 | | |
| B | 0.038 | 0.0261 | 32 | 0.8338 | | |
| C | 0.073 | 0.0582 | 16 | 0.9310 | | |
| D | 0.175 | 0.1526 | 8 | 1.2207 | Average | 1.082 |
| E | 0.373 | 0.3361 | 4 | 1.3444 | STDEV | 0.240 |
| F | 0.653 | 0.5950 | 2 | 1.1901 | %RSD | 22.15 |
| G | 0.854 | 0.7818 | 1 | 0.7818 | Horrat | 1.04 |

| M-Ctrl-D1-2-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.030 | 0.0181 | 64 | 1.1610 | | |
| B | 0.048 | 0.0348 | 32 | 1.1123 | | |
| C | 0.084 | 0.0686 | 16 | 1.0984 | | |
| D | 0.189 | 0.1657 | 8 | 1.3255 | Average | 1.219 |
| E | 0.387 | 0.3491 | 4 | 1.3963 | STDEV | 0.134 |
| F | 0.659 | 0.6006 | 2 | 1.2012 | %RSD | 11.01 |
| G | 0.870 | 0.7957 | 1 | 0.7957 | Horrat | 0.52 |

| M-Ctrl-D1-2-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.035 | 0.0232 | 64 | 1.4840 | | |
| B | 0.049 | 0.0356 | 32 | 1.1405 | | |
| C | 0.093 | 0.0767 | 16 | 1.2265 | | |
| D | 0.194 | 0.1705 | 8 | 1.3636 | Average | 1.348 |
| E | 0.423 | 0.3819 | 4 | 1.5276 | STDEV | 0.165 |
| F | 0.685 | 0.6251 | 2 | 1.2503 | %RSD | 12.23 |
| G | 0.858 | 0.7847 | 1 | 0.7847 | Horrat | 0.57 |

| M-F2-D1-2-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.071 | 0.0565 | 64 | 3.6143 | | |
| B | 0.130 | 0.1109 | 32 | 3.5479 | | |
| C | 0.301 | 0.2693 | 16 | 4.3080 | | |
| D | 0.559 | 0.5086 | 8 | 4.0685 | Average | 3.885 |
| E | 0.766 | 0.7000 | 4 | 2.8000 | STDEV | 0.365 |
| F | 0.895 | 0.8190 | 2 | 1.6379 | %RSD | 9.39 |
| G | 0.996 | 0.9130 | 1 | 0.9130 | Horrat | 0.44 |

| M-Ctrl-D1-1-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.035 | 0.0231 | 64 | 1.478 | | |
| B | 0.043 | 0.0302 | 32 | 0.966 | | |
| C | 0.083 | 0.0675 | 16 | 1.081 | | |
| D | 0.166 | 0.1438 | 8 | 1.151 | Average | 1.215 |
| E | 0.388 | 0.3494 | 4 | 1.398 | STDEV | 0.216 |
| F | 0.646 | 0.5892 | 2 | 1.178 | %RSD | 17.8 |
| G | 0.862 | 0.7886 | 1 | 0.789 | Horrat | 0.83 |

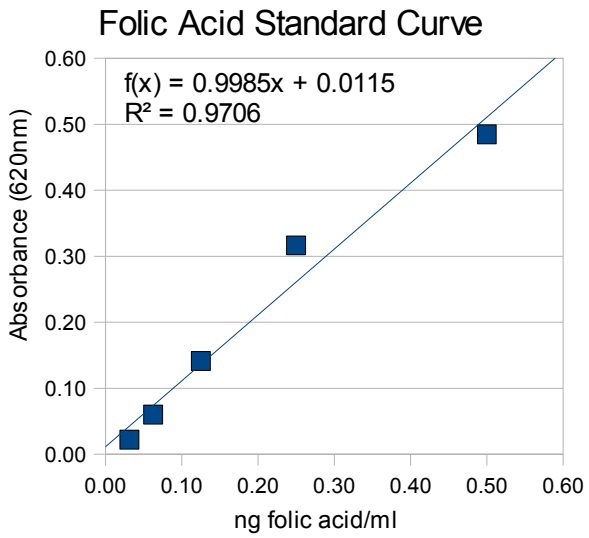
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-3 Plate 1 | M-Ctrl-D2-1-a | 1.0741 | 2 | 1.266 | 0.1266 | 1 | 0.1179 | 54.17 | 0.257 | n/a | n/a |
| | M-F2-D2-2-a | 1.0055 | | 1.378 | 0.1378 | 2.5 | 0.3426 | 52.49 | 0.721 | 3.476 | 20.74 |
| | M-Ctrl-D2-2-b | 1.0118 | | 1.149 | 0.1149 | 1 | 0.1135 | 54.59 | 0.250 | n/a | n/a |
| | M-F2-D2-1-a | 1.0145 | | 2.031 | 0.2031 | 2.5 | 0.5006 | 52.85 | 1.062 | 3.501 | 30.33 |
| | M-F1-D2-2-b | 1.0306 | | 2.156 | 0.2156 | 2.5 | 0.5231 | 56.04 | 1.190 | 3.567 | 33.36 |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.025 | 0.019 | 0.015 | 0.014 | 0.024 | 0.027 | 0.021 | 0.028 | 0.029 | 0.035 | 0.050 | 0.038 |
| B | 0.060 | 0.060 | 0.035 | 0.032 | 0.047 | 0.049 | 0.034 | 0.036 | 0.066 | 0.073 | 0.068 | 0.081 |
| C | 0.130 | 0.153 | 0.076 | 0.077 | 0.086 | 0.108 | 0.063 | 0.071 | 0.133 | 0.173 | 0.149 | 0.131 |
| D | 0.313 | 0.320 | 0.214 | 0.194 | 0.226 | 0.216 | 0.155 | 0.150 | 0.284 | 0.272 | 0.325 | 0.319 |
| E | 0.509 | 0.461 | 0.330 | 0.345 | 0.398 | 0.344 | 0.319 | 0.308 | 0.470 | 0.479 | 0.565 | 0.530 |
| F | 0.729 | 0.712 | 0.593 | 0.607 | 0.628 | 0.636 | 0.613 | 0.518 | 0.635 | 0.666 | 0.694 | 0.707 |
| G | 0.793 | 0.762 | 0.752 | 0.800 | 0.792 | 0.768 | 0.759 | 0.732 | 0.778 | 0.832 | 0.840 | 0.819 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | M-Ctrl-D2-1-a | M-F2-D2-2-a | M-Ctrl-D2-2-b | M-F2-D2-1-a | M-F1-D2-2-b |
|---|---|---|---|---|---|---|
| A | 0.022 | 0.014 | 0.025 | 0.024 | 0.032 | 0.044 |
| B | 0.060 | 0.034 | 0.048 | 0.035 | 0.070 | 0.074 |
| C | 0.141 | 0.076 | 0.097 | 0.067 | 0.153 | 0.140 |
| D | 0.316 | 0.204 | 0.221 | 0.152 | 0.278 | 0.322 |
| E | 0.485 | 0.337 | 0.371 | 0.313 | 0.475 | 0.547 |
| F | 0.720 | 0.600 | 0.632 | 0.565 | 0.650 | 0.700 |
| G | 0.777 | 0.776 | 0.780 | 0.746 | 0.805 | 0.829 |




| M-Ctrl-D2-1-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.014 | 0.0026 | 64 | 0.1675 | | |
| B | 0.034 | 0.0221 | 32 | 0.7087 | | |
| C | 0.076 | 0.0651 | 16 | 1.0410 | | |
| D | 0.204 | 0.1924 | 8 | 1.5388 | Average | 1.266 |
| E | 0.337 | 0.3265 | 4 | 1.3058 | STDEV | 0.212 |
| F | 0.600 | 0.5892 | 2 | 1.1785 | %RSD | 16.71 |
| G | 0.776 | 0.7659 | 1 | 0.7659 | Horrat | 0.78 |

| M-F2-D2-2-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.025 | 0.0136 | 64 | 0.8726 | | |
| B | 0.048 | 0.0364 | 32 | 1.1638 | | |
| C | 0.097 | 0.0853 | 16 | 1.3647 | | |
| D | 0.221 | 0.2097 | 8 | 1.6778 | Average | 1.378 |
| E | 0.371 | 0.3601 | 4 | 1.4402 | STDEV | 0.199 |
| F | 0.632 | 0.6214 | 2 | 1.2428 | %RSD | 14.43 |
| G | 0.780 | 0.7699 | 1 | 0.7699 | Horrat | 0.68 |

| M-F2-D2-1-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.032 | 0.0203 | 64 | 1.2988 | | |
| B | 0.070 | 0.0584 | 32 | 1.8688 | | |
| C | 0.153 | 0.1416 | 16 | 2.2660 | | |
| D | 0.278 | 0.2669 | 8 | 2.1353 | Average | 2.031 |
| E | 0.475 | 0.4639 | 4 | 1.8554 | STDEV | 0.203 |
| F | 0.650 | 0.6398 | 2 | 1.2796 | %RSD | 9.98 |
| G | 0.805 | 0.7948 | 1 | 0.7948 | Horrat | 0.47 |

| M-Ctrl-D2-2-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.024 | 0.0128 | 64 | 0.8181 | | |
| B | 0.035 | 0.0234 | 32 | 0.7504 | | |
| C | 0.067 | 0.0555 | 16 | 0.8887 | | |
| D | 0.152 | 0.1411 | 8 | 1.1286 | Average | 1.149 |
| E | 0.313 | 0.3023 | 4 | 1.2091 | STDEV | 0.053 |
| F | 0.565 | 0.5544 | 2 | 1.1088 | %RSD | 4.62 |
| G | 0.746 | 0.7352 | 1 | 0.7352 | Horrat | 0.22 |

| M-F1-D2-2-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.044 | 0.0324 | 64 | 2.071 | | |
| B | 0.074 | 0.0630 | 32 | 2.016 | | |
| C | 0.140 | 0.1286 | 16 | 2.058 | | |
| D | 0.322 | 0.3111 | 8 | 2.489 | Average | 2.156 |
| E | 0.547 | 0.5368 | 4 | 2.147 | STDEV | 0.192 |
| F | 0.700 | 0.6896 | 2 | 1.379 | %RSD | 8.9 |
| G | 0.829 | 0.8190 | 1 | 0.819 | Horrat | 0.42 |

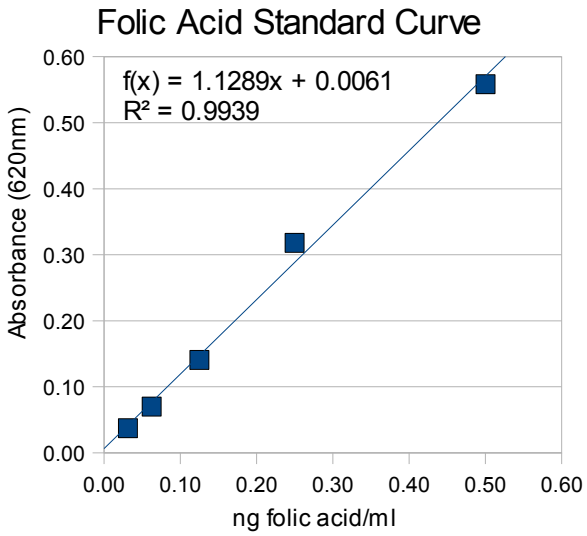
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-3 Plate 2 | M-F1-D2-2-a | 1.0157 | 2 | 2.007 | 0.2007 | 2.5 | 0.4941 | 55.92 | 1.121 | 3.558 | 31.51 |
| | M-Ctrl-D2-2-a | 1.0192 | | 1.003 | 0.1003 | 1 | 0.0984 | 54.38 | 0.216 | n/a | n/a |
| | M-F2-D2-1-b | 1.0112 | | 1.830 | 0.1830 | 2.5 | 0.4523 | 52.75 | 0.957 | 3.494 | 27.4 |
| | M-Ctrl-D2-1-b | 1.0018 | | 1.238 | 0.1238 | 1 | 0.1236 | 54.28 | 0.270 | n/a | n/a |
| | M-F2-D2-2-b | 1.0292 | | 1.375 | 0.1375 | 2.5 | 0.3339 | 52.82 | 0.708 | 3.499 | 20.23 |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.038 | 0.036 | 0.042 | 0.038 | 0.031 | 0.026 | 0.035 | 0.041 | 0.029 | 0.035 | 0.040 | 0.030 |
| B | 0.067 | 0.072 | 0.072 | 0.071 | 0.047 | 0.050 | 0.064 | 0.064 | 0.047 | 0.054 | 0.052 | 0.042 |
| C | 0.135 | 0.146 | 0.131 | 0.154 | 0.070 | 0.060 | 0.132 | 0.145 | 0.085 | 0.088 | 0.098 | 0.094 |
| D | 0.334 | 0.301 | 0.347 | 0.335 | 0.144 | 0.156 | 0.282 | 0.282 | 0.188 | 0.193 | 0.222 | 0.205 |
| E | 0.568 | 0.549 | 0.560 | 0.552 | 0.290 | 0.308 | 0.514 | 0.547 | 0.359 | 0.355 | 0.450 | 0.460 |
| F | 0.687 | 0.734 | 0.745 | 0.743 | 0.635 | 0.644 | 0.712 | 0.697 | 0.620 | 0.592 | 0.662 | 0.681 |
| G | 0.825 | 0.849 | 0.858 | 0.827 | 0.751 | 0.832 | 0.894 | 0.873 | 0.776 | 0.817 | 0.835 | 0.807 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | M-F1-D2-2-a | M-Ctrl-D2-2-a | M-F2-D2-1-b | M-Ctrl-D2-1-b | M-F2-D2-2-b |
|---|---|---|---|---|---|---|
| A | 0.037 | 0.040 | 0.029 | 0.038 | 0.032 | 0.035 |
| B | 0.070 | 0.072 | 0.049 | 0.064 | 0.051 | 0.047 |
| C | 0.141 | 0.143 | 0.065 | 0.138 | 0.087 | 0.096 |
| D | 0.318 | 0.341 | 0.150 | 0.282 | 0.190 | 0.214 |
| E | 0.559 | 0.556 | 0.299 | 0.530 | 0.357 | 0.455 |
| F | 0.710 | 0.744 | 0.639 | 0.704 | 0.606 | 0.672 |
| G | 0.837 | 0.843 | 0.792 | 0.883 | 0.797 | 0.821 |




| M-F1-D2-2-a | | | | | |
|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | |
| A | 0.040 | 0.0300 | 64 | 1.9184 | |
| B | 0.072 | 0.0583 | 32 | 1.8648 | |
| C | 0.143 | 0.1209 | 16 | 1.9336 | |
| D | 0.341 | 0.2965 | 8 | 2.3720 | Average 2.007 |
| E | 0.556 | 0.4871 | 4 | 1.9486 | STDEV 0.206 |
| F | 0.744 | 0.6536 | 2 | 1.3072 | %RSD 10.27 |
| G | 0.843 | 0.7409 | 1 | 0.7409 | Horrat 0.48 |

| M-Ctrl-D2-2-a | | | | | |
|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | |
| A | 0.029 | 0.0200 | 64 | 1.2807 | |
| B | 0.049 | 0.0376 | 32 | 1.2044 | |
| C | 0.065 | 0.0520 | 16 | 0.8318 | |
| D | 0.150 | 0.1277 | 8 | 1.0214 | Average 1.003 |
| E | 0.299 | 0.2592 | 4 | 1.0370 | STDEV 0.122 |
| F | 0.639 | 0.5608 | 2 | 1.1215 | %RSD 12.19 |
| G | 0.792 | 0.6957 | 1 | 0.6957 | Horrat 0.57 |

| M-Ctrl-D2-1-b | | | | | |
|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | |
| A | 0.032 | 0.0229 | 64 | 1.4677 | |
| B | 0.051 | 0.0394 | 32 | 1.2611 | |
| C | 0.087 | 0.0713 | 16 | 1.1414 | |
| D | 0.190 | 0.1633 | 8 | 1.3066 | Average 1.238 |
| E | 0.357 | 0.3110 | 4 | 1.2441 | STDEV 0.070 |
| F | 0.606 | 0.5317 | 2 | 1.0635 | %RSD 5.63 |
| G | 0.797 | 0.7001 | 1 | 0.7001 | Horrat 0.26 |

| M-F2-D2-1-b | | | | | |
|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | |
| A | 0.038 | 0.0283 | 64 | 1.8107 | |
| B | 0.064 | 0.0517 | 32 | 1.6536 | |
| C | 0.138 | 0.1170 | 16 | 1.8713 | |
| D | 0.282 | 0.2443 | 8 | 1.9542 | Average 1.830 |
| E | 0.530 | 0.4644 | 4 | 1.8577 | STDEV 0.111 |
| F | 0.704 | 0.6184 | 2 | 1.2368 | %RSD 6.07 |
| G | 0.883 | 0.7769 | 1 | 0.7769 | Horrat 0.28 |

| M-F2-D2-2-b | | | | | |
|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | |
| A | 0.035 | 0.0255 | 64 | 1.635 | |
| B | 0.047 | 0.0364 | 32 | 1.166 | |
| C | 0.096 | 0.0794 | 16 | 1.270 | |
| D | 0.214 | 0.1838 | 8 | 1.470 | Average 1.375 |
| E | 0.455 | 0.3979 | 4 | 1.591 | STDEV 0.192 |
| F | 0.672 | 0.5895 | 2 | 1.179 | %RSD 13.96 |
| G | 0.821 | 0.7219 | 1 | 0.722 | Horrat 0.65 |

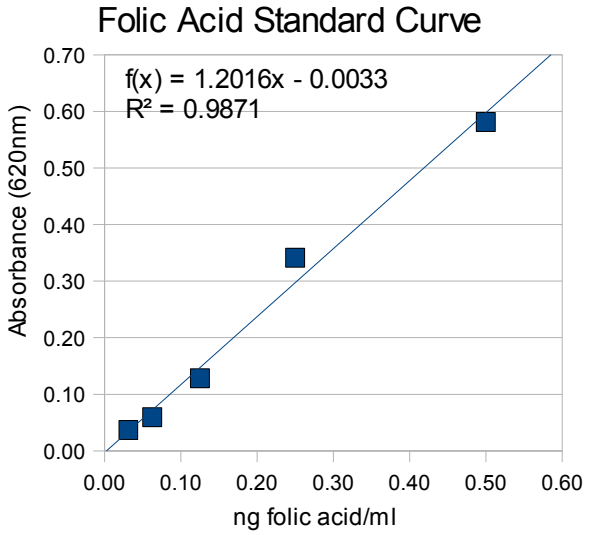
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-3 Plate 3 | M-F1-D2-1-a | 1.0358 | 2 | 2.382 | 0.2382 | 2.5 | 0.5750 | 55.21 | 1.284 | 3.505 | 36.63 |
| | M-F1-D2-1-b | 1.0474 | | 2.410 | 0.2410 | 2.5 | 0.5752 | 55.38 | 1.289 | 3.517 | 36.65 |
| | T-Ctrl-D1-12a-a | 1.0777 | | 0.447 | 0.0447 | 2.5 | 0.1037 | 49.81 | 0.207 | n/a | n/a |
| | T-F1-D1-12a-a | 1.0030 | | 4.998 | 0.4998 | 2.5 | 1.2457 | 44.44 | 2.242 | 3.612 | 62.07 |
| | T-F2-D1-12a-a | 1.0301 | | 3.510 | 0.3510 | 2.5 | 0.8519 | 48.2 | 1.645 | 3.517 | 46.77 |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.038 | 0.036 | 0.045 | 0.042 | 0.055 | 0.046 | 0.026 | 0.032 | 0.099 | 0.100 | 0.067 | 0.075 |
| B | 0.059 | 0.061 | 0.071 | 0.077 | 0.085 | 0.083 | 0.029 | 0.031 | 0.163 | 0.169 | 0.112 | 0.092 |
| C | 0.132 | 0.125 | 0.181 | 0.209 | 0.204 | 0.179 | 0.044 | 0.043 | 0.407 | 0.430 | 0.295 | 0.295 |
| D | 0.352 | 0.331 | 0.405 | 0.401 | 0.384 | 0.371 | 0.066 | 0.061 | 0.634 | 0.677 | 0.444 | 0.545 |
| E | 0.561 | 0.601 | 0.648 | 0.621 | 0.642 | 0.663 | 0.134 | 0.115 | 0.805 | 0.797 | 0.784 | 0.773 |
| F | 0.771 | 0.764 | 0.810 | 0.832 | 0.801 | 0.792 | 0.284 | 0.269 | 0.936 | 0.957 | 0.901 | 0.883 |
| G | 0.818 | 0.883 | 0.910 | 0.930 | 0.915 | 0.914 | 0.537 | 0.543 | 1.048 | 1.041 | 0.975 | 1.003 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | M-F1-D2-1-a | M-F1-D2-1-b | T-Ctrl-D1-12a-a | T-F1-D1-12a-a | T-F2-D1-12a-a |
|---|---|---|---|---|---|---|
| A | 0.037 | 0.044 | 0.050 | 0.029 | 0.100 | 0.071 |
| B | 0.060 | 0.074 | 0.084 | 0.030 | 0.166 | 0.102 |
| C | 0.128 | 0.195 | 0.192 | 0.044 | 0.419 | 0.295 |
| D | 0.341 | 0.403 | 0.378 | 0.063 | 0.656 | 0.494 |
| E | 0.581 | 0.635 | 0.653 | 0.125 | 0.801 | 0.779 |
| F | 0.768 | 0.821 | 0.796 | 0.277 | 0.947 | 0.892 |
| G | 0.850 | 0.920 | 0.914 | 0.540 | 1.044 | 0.989 |




M-F1-D2-1-a

| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
|---|---|---|---|---|---|---|
| A | 0.044 | 0.0392 | 64 | 2.5074 | | |
| B | 0.074 | 0.0643 | 32 | 2.0577 | | |
| C | 0.195 | 0.1652 | 16 | 2.6435 | | |
| D | 0.403 | 0.3382 | 8 | 2.7058 | Average | 2.382 |
| E | 0.635 | 0.5307 | 4 | 2.1226 | STDEV | 0.339 |
| F | 0.821 | 0.6860 | 2 | 1.3719 | %RSD | 14.25 |
| G | 0.920 | 0.7683 | 1 | 0.7683 | Horrat | 0.67 |

M-F1-D2-1-b

| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
|---|---|---|---|---|---|---|
| A | 0.050 | 0.0447 | 64 | 2.8588 | | |
| B | 0.084 | 0.0727 | 32 | 2.3279 | | |
| C | 0.192 | 0.1621 | 16 | 2.5936 | | |
| D | 0.378 | 0.3169 | 8 | 2.5354 | Average | 2.410 |
| E | 0.653 | 0.5458 | 4 | 2.1832 | STDEV | 0.189 |
| F | 0.796 | 0.6651 | 2 | 1.3302 | %RSD | 7.86 |
| G | 0.914 | 0.7636 | 1 | 0.7636 | Horrat | 0.37 |

T-F1-D1-12a-a

| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
|---|---|---|---|---|---|---|
| A | 0.100 | 0.0856 | 64 | 5.4811 | | |
| B | 0.166 | 0.1409 | 32 | 4.5083 | | |
| C | 0.419 | 0.3510 | 16 | 5.6165 | | |
| D | 0.656 | 0.5481 | 8 | 4.3850 | Average | 4.998 |
| E | 0.801 | 0.6691 | 4 | 2.6765 | STDEV | 0.641 |
| F | 0.947 | 0.7903 | 2 | 1.5805 | %RSD | 12.82 |
| G | 1.044 | 0.8716 | 1 | 0.8716 | Horrat | 0.60 |

T-Ctrl-D1-12a-a

| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
|---|---|---|---|---|---|---|
| A | 0.029 | 0.0269 | 64 | 1.7220 | | |
| B | 0.030 | 0.0279 | 32 | 0.8943 | | |
| C | 0.044 | 0.0390 | 16 | 0.6242 | | |
| D | 0.063 | 0.0555 | 8 | 0.4442 | Average | 0.447 |
| E | 0.125 | 0.1065 | 4 | 0.4259 | STDEV | 0.017 |
| F | 0.277 | 0.2328 | 2 | 0.4656 | %RSD | 3.71 |
| G | 0.540 | 0.4522 | 1 | 0.4522 | Horrat | 0.17 |

T-F2-D1-12a-a

| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
|---|---|---|---|---|---|---|
| A | 0.071 | 0.0618 | 64 | 3.956 | | |
| B | 0.102 | 0.0876 | 32 | 2.803 | | |
| C | 0.295 | 0.2481 | 16 | 3.970 | | |
| D | 0.494 | 0.4140 | 8 | 3.312 | Average | 3.510 |
| E | 0.779 | 0.6505 | 4 | 2.602 | STDEV | 0.562 |
| F | 0.892 | 0.7449 | 2 | 1.490 | %RSD | 16.02 |
| G | 0.989 | 0.8258 | 1 | 0.826 | Horrat | 0.75 |

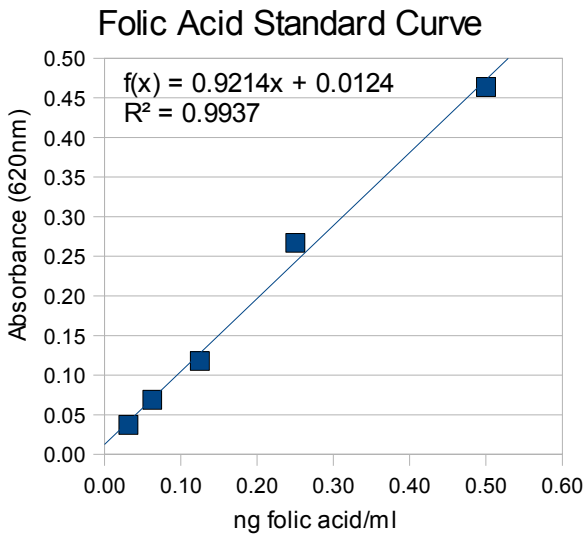
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-4 Plate 1 | T-F2-D2-12a-b | 1.0226 | 2 | 3.167 | 0.3167 | 2.5 | 0.7742 | 43.03 | 1.359 | 3.530 | 38.5 |
| | T-F2-D2-12a-a | 1.0128 | | 2.814 | 0.2814 | 2.5 | 0.6945 | 44.12 | 1.243 | 3.530 | 35.21 |
| | T-F1-D2-12a-b | 1.0189 | | 3.823 | 0.3823 | 2.5 | 0.9380 | 45.71 | 1.728 | 3.510 | 49.23 |
| | T-Ctrl-D2-12a-b | 1.0223 | | 1.133 | 0.1133 | 1 | 0.1108 | 42.67 | 0.193 | n/a | n/a |
| | T-Ctrl-D1-12a-b | 1.0313 | | 1.195 | 0.1195 | 1 | 0.1158 | 42.56 | 0.202 | n/a | n/a |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.039 | 0.035 | 0.045 | 0.045 | 0.050 | 0.042 | 0.059 | 0.061 | 0.025 | 0.038 | 0.034 | 0.036 |
| B | 0.077 | 0.061 | 0.102 | 0.102 | 0.100 | 0.088 | 0.131 | 0.131 | 0.038 | 0.050 | 0.039 | 0.037 |
| C | 0.132 | 0.103 | 0.217 | 0.173 | 0.301 | 0.200 | 0.250 | 0.262 | 0.064 | 0.080 | 0.076 | 0.082 |
| D | 0.276 | 0.258 | 0.372 | 0.395 | 0.403 | 0.373 | 0.398 | 0.462 | 0.143 | 0.148 | 0.163 | 0.154 |
| E | 0.462 | 0.465 | 0.609 | 0.547 | 0.531 | 0.571 | 0.651 | 0.600 | 0.295 | 0.304 | 0.335 | 0.357 |
| F | 0.663 | 0.621 | 0.751 | 0.764 | 0.740 | 0.755 | 0.804 | 0.771 | 0.569 | 0.564 | 0.601 | 0.643 |
| G | 0.666 | 0.729 | 0.841 | 0.798 | 0.828 | 0.795 | 0.829 | 0.852 | 0.708 | 0.753 | 0.803 | 0.753 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | T-F2-D2-12a-b | T-F2-D2-12a-a | T-F1-D2-12a-b | T-Ctrl-D2-12a-b | T-Ctrl-D1-12a-b |
|---|---|---|---|---|---|---|
| A | 0.037 | 0.045 | 0.046 | 0.060 | 0.032 | 0.035 |
| B | 0.069 | 0.102 | 0.094 | 0.131 | 0.044 | 0.038 |
| C | 0.118 | 0.195 | 0.251 | 0.256 | 0.072 | 0.079 |
| D | 0.267 | 0.383 | 0.388 | 0.430 | 0.145 | 0.159 |
| E | 0.464 | 0.578 | 0.551 | 0.626 | 0.299 | 0.346 |
| F | 0.642 | 0.757 | 0.747 | 0.788 | 0.567 | 0.622 |
| G | 0.697 | 0.820 | 0.811 | 0.841 | 0.731 | 0.778 |




| T-F2-D2-12a-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.045 | 0.0350 | 64 | 2.2425 | | |
| B | 0.102 | 0.0973 | 32 | 3.1130 | | |
| C | 0.195 | 0.1979 | 16 | 3.1662 | | |
| D | 0.383 | 0.4026 | 8 | 3.2211 | Average | 3.167 |
| E | 0.578 | 0.6143 | 4 | 2.4571 | STDEV | 0.054 |
| F | 0.757 | 0.8085 | 2 | 1.6170 | %RSD | 1.71 |
| G | 0.820 | 0.8763 | 1 | 0.8763 | Horrat | 0.08 |

| T-F2-D2-12a-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.046 | 0.0366 | 64 | 2.3432 | | |
| B | 0.094 | 0.0887 | 32 | 2.8369 | | |
| C | 0.251 | 0.2587 | 16 | 4.1387 | | |
| D | 0.388 | 0.4076 | 8 | 3.2606 | Average | 2.814 |
| E | 0.551 | 0.5844 | 4 | 2.3377 | STDEV | 0.459 |
| F | 0.747 | 0.7975 | 2 | 1.5950 | %RSD | 16.32 |
| G | 0.811 | 0.8670 | 1 | 0.8670 | Horrat | 0.76 |

| T-Ctrl-D2-12a-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.032 | 0.0208 | 64 | 1.3291 | | |
| B | 0.044 | 0.0342 | 32 | 1.0952 | | |
| C | 0.072 | 0.0647 | 16 | 1.0347 | | |
| D | 0.145 | 0.1444 | 8 | 1.1551 | Average | 1.133 |
| E | 0.299 | 0.3113 | 4 | 1.2452 | STDEV | 0.090 |
| F | 0.567 | 0.6016 | 2 | 1.2031 | %RSD | 7.93 |
| G | 0.731 | 0.7794 | 1 | 0.7794 | Horrat | 0.37 |

| T-F1-D2-12a-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.060 | 0.0516 | 64 | 3.3052 | | |
| B | 0.131 | 0.1289 | 32 | 4.1254 | | |
| C | 0.256 | 0.2646 | 16 | 4.2333 | | |
| D | 0.430 | 0.4534 | 8 | 3.6274 | Average | 3.823 |
| E | 0.626 | 0.6656 | 4 | 2.6624 | STDEV | 0.434 |
| F | 0.788 | 0.8414 | 2 | 1.6827 | %RSD | 11.36 |
| G | 0.841 | 0.8990 | 1 | 0.8990 | Horrat | 0.53 |

| T-Ctrl-D1-12a-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.035 | 0.0249 | 64 | 1.593 | | |
| B | 0.038 | 0.0281 | 32 | 0.899 | | |
| C | 0.079 | 0.0725 | 16 | 1.160 | | |
| D | 0.159 | 0.1590 | 8 | 1.272 | Average | 1.195 |
| E | 0.346 | 0.3621 | 4 | 1.448 | STDEV | 0.230 |
| F | 0.622 | 0.6616 | 2 | 1.323 | %RSD | 19.27 |
| G | 0.778 | 0.8313 | 1 | 0.831 | Horrat | 0.9 |

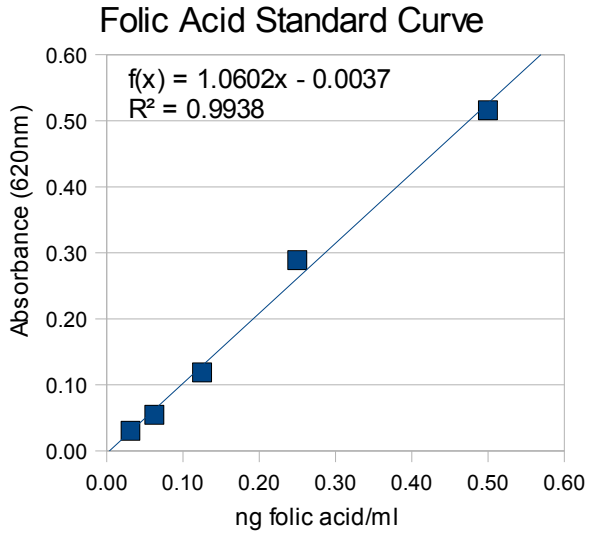
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-4 Plate 2 | T-F1-D1-12f-a | 1.0103 | 2 | 5.361 | 0.5361 | 2.5 | 1.3266 | 42.8 | 2.319 | 3.612 | 64.2 |
| | T-Ctrl-D1-12f-a | 1.0114 | | 1.548 | 0.1548 | 1 | 0.1530 | 38.81 | 0.250 | n/a | n/a |
| | T-F2-D1-12a-b | 1.0208 | | 3.709 | 0.3709 | 2.5 | 0.9084 | 40.8 | 1.534 | 3.517 | 43.63 |
| | T-F1-D1-12a-b | 1.0120 | | 5.600 | 0.5600 | 2.5 | 1.3835 | 39.53 | 2.288 | 3.612 | 63.34 |
| | T-Ctrl-D2-12a-a | 1.0112 | | 1.156 | 0.1156 | 1 | 0.1143 | 42.11 | 0.197 | n/a | n/a |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.028 | 0.033 | 0.086 | 0.086 | 0.025 | 0.030 | 0.062 | 0.064 | 0.090 | 0.095 | 0.033 | 0.038 |
| B | 0.056 | 0.053 | 0.175 | 0.174 | 0.040 | 0.056 | 0.117 | 0.119 | 0.185 | 0.175 | 0.034 | 0.027 |
| C | 0.118 | 0.120 | 0.346 | 0.347 | 0.084 | 0.101 | 0.257 | 0.243 | 0.356 | 0.360 | 0.072 | 0.070 |
| D | 0.310 | 0.268 | 0.634 | 0.667 | 0.205 | 0.203 | 0.444 | 0.512 | 0.584 | 0.591 | 0.151 | 0.162 |
| E | 0.518 | 0.514 | 0.709 | 0.747 | 0.401 | 0.438 | 0.648 | 0.585 | 0.729 | 0.751 | 0.329 | 0.340 |
| F | 0.645 | 0.679 | 0.842 | 0.861 | 0.643 | 0.661 | 0.787 | 0.752 | 0.878 | 0.865 | 0.598 | 0.603 |
| G | 0.772 | 0.783 | 0.940 | 0.945 | 0.823 | 0.813 | 0.907 | 0.904 | 0.943 | 0.934 | 0.752 | 0.747 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | T-F1-D1-12f-a | T-Ctrl-D1-12f-a | T-F2-D1-12a-b | T-F1-D1-12a-b | T-Ctrl-D2-12a-a |
|---|---|---|---|---|---|---|
| A | 0.030 | 0.086 | 0.027 | 0.063 | 0.092 | 0.036 |
| B | 0.054 | 0.175 | 0.048 | 0.118 | 0.180 | 0.030 |
| C | 0.119 | 0.346 | 0.093 | 0.250 | 0.358 | 0.071 |
| D | 0.289 | 0.650 | 0.204 | 0.478 | 0.587 | 0.156 |
| E | 0.516 | 0.728 | 0.420 | 0.617 | 0.740 | 0.335 |
| F | 0.662 | 0.851 | 0.652 | 0.769 | 0.872 | 0.600 |
| G | 0.777 | 0.942 | 0.818 | 0.906 | 0.939 | 0.749 |




| T-F1-D1-12f-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.086 | 0.0846 | 64 | 5.4169 | | |
| B | 0.175 | 0.1682 | 32 | 5.3832 | | |
| C | 0.346 | 0.3302 | 16 | 5.2833 | | |
| D | 0.650 | 0.6171 | 8 | 4.9367 | Average | 5.361 |
| E | 0.728 | 0.6903 | 4 | 2.7612 | STDEV | 0.069 |
| F | 0.851 | 0.8068 | 2 | 1.6135 | %RSD | 1.30 |
| G | 0.942 | 0.8926 | 1 | 0.8926 | Horrat | 0.06 |

| T-Ctrl-D1-12f-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.027 | 0.0294 | 64 | 1.8818 | | |
| B | 0.048 | 0.0490 | 32 | 1.5688 | | |
| C | 0.093 | 0.0910 | 16 | 1.4561 | | |
| D | 0.204 | 0.1961 | 8 | 1.5692 | Average | 1.548 |
| E | 0.420 | 0.3993 | 4 | 1.5970 | STDEV | 0.063 |
| F | 0.652 | 0.6185 | 2 | 1.2369 | %RSD | 4.04 |
| G | 0.818 | 0.7753 | 1 | 0.7753 | Horrat | 0.19 |

| T-F1-D1-12a-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.092 | 0.0905 | 64 | 5.7942 | | |
| B | 0.180 | 0.1732 | 32 | 5.5432 | | |
| C | 0.358 | 0.3415 | 16 | 5.4637 | | |
| D | 0.587 | 0.5574 | 8 | 4.4594 | Average | 5.600 |
| E | 0.740 | 0.7016 | 4 | 2.8063 | STDEV | 0.173 |
| F | 0.872 | 0.8258 | 2 | 1.6516 | %RSD | 3.08 |
| G | 0.939 | 0.8891 | 1 | 0.8891 | Horrat | 0.14 |

| T-F2-D1-12a-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.063 | 0.0629 | 64 | 4.0252 | | |
| B | 0.118 | 0.1145 | 32 | 3.6654 | | |
| C | 0.250 | 0.2393 | 16 | 3.8282 | | |
| D | 0.478 | 0.4542 | 8 | 3.6337 | Average | 3.709 |
| E | 0.617 | 0.5853 | 4 | 2.3410 | STDEV | 0.104 |
| F | 0.769 | 0.7294 | 2 | 1.4588 | %RSD | 2.81 |
| G | 0.906 | 0.8580 | 1 | 0.8580 | Horrat | 0.13 |

| T-Ctrl-D2-12a-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.036 | 0.0371 | 64 | 2.374 | | |
| B | 0.030 | 0.0322 | 32 | 1.030 | | |
| C | 0.071 | 0.0703 | 16 | 1.124 | | |
| D | 0.156 | 0.1511 | 8 | 1.209 | Average | 1.156 |
| E | 0.335 | 0.3192 | 4 | 1.277 | STDEV | 0.093 |
| F | 0.600 | 0.5697 | 2 | 1.139 | %RSD | 8.04 |
| G | 0.749 | 0.7105 | 1 | 0.711 | Horrat | 0.38 |

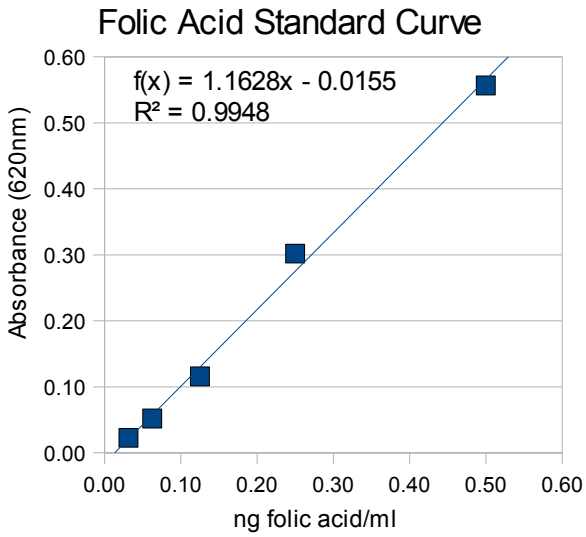
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-4 Plate 3 | T-F1-D2-12a-a | 1.0134 | 2 | 3.747 | 0.3747 | 2.5 | 0.9244 | 44.46 | 1.664 | 3.510 | 47.42 |
| | T-F2-D1-12f-a | 1.0221 | | 3.622 | 0.3622 | 2.5 | 0.8860 | 44.04 | 1.583 | 3.517 | 45.02 |
| | | | | | | | | | | | |
| | T-F2-D1-12f-b | 1.0469 | | 4.220 | 0.4220 | 2.5 | 1.0078 | 50.9 | 2.053 | 3.517 | 58.37 |
| | T-Ctrl-D1-12f-b | 1.0147 | | 1.591 | 0.1591 | 1 | 0.1568 | 41.43 | 0.268 | n/a | n/a |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.022 | 0.023 | 0.051 | 0.057 | 0.061 | 0.066 | 0.103 | 0.625 | 0.074 | 0.088 | 0.048 | 0.045 |
| B | 0.052 | 0.052 | 0.113 | 0.119 | 0.098 | 0.122 | 0.203 | 0.362 | 0.138 | 0.148 | 0.052 | 0.048 |
| C | 0.115 | 0.116 | 0.263 | 0.261 | 0.264 | 0.261 | 0.380 | 0.488 | 0.308 | 0.327 | 0.087 | 0.092 |
| D | 0.304 | 0.300 | 0.530 | 0.526 | 0.483 | 0.532 | 0.677 | 0.509 | 0.523 | 0.525 | 0.229 | 0.209 |
| E | 0.566 | 0.547 | 0.723 | 0.685 | 0.673 | 0.646 | 0.775 | 0.795 | 0.703 | 0.702 | 0.394 | 0.449 |
| F | 0.721 | 0.685 | 0.801 | 0.838 | 0.868 | 0.818 | 0.895 | 0.914 | 0.853 | 0.834 | 0.706 | 0.711 |
| G | 0.817 | 0.803 | 0.933 | 0.889 | 0.907 | 0.918 | 0.944 | 0.940 | 0.912 | 0.934 | 0.851 | 0.813 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | T-F1-D2-12a-a | T-F2-D1-12f-a | T-F1-D1-12f-b | T-F2-D1-12f-b | T-Ctrl-D1-12f-b |
|---|---|---|---|---|---|---|
| A | 0.023 | 0.054 | 0.063 | 0.364 | 0.081 | 0.047 |
| B | 0.052 | 0.116 | 0.110 | 0.283 | 0.143 | 0.050 |
| C | 0.116 | 0.262 | 0.262 | 0.439 | 0.318 | 0.090 |
| D | 0.302 | 0.528 | 0.507 | 0.638 | 0.524 | 0.219 |
| E | 0.557 | 0.704 | 0.659 | 0.785 | 0.703 | 0.422 |
| F | 0.703 | 0.820 | 0.843 | 0.904 | 0.844 | 0.708 |
| G | 0.810 | 0.911 | 0.913 | 0.942 | 0.923 | 0.832 |




| T-F1-D2-12a-a | | | | | |
|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | |
| A | 0.054 | 0.0597 | 64 | 3.8235 | |
| B | 0.116 | 0.1130 | 32 | 3.6149 | |
| C | 0.262 | 0.2382 | 16 | 3.8112 | |
| D | 0.528 | 0.4673 | 8 | 3.7381 | Average 3.747 |
| E | 0.704 | 0.6188 | 4 | 2.4753 | STDEV 0.096 |
| F | 0.820 | 0.7180 | 2 | 1.4360 | %RSD 2.56 |
| G | 0.911 | 0.7964 | 1 | 0.7964 | Horrat 0.12 |

| T-F2-D1-12f-a | | | | | |
|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | |
| A | 0.063 | 0.0677 | 64 | 4.3353 | |
| B | 0.110 | 0.1078 | 32 | 3.4485 | |
| C | 0.262 | 0.2389 | 16 | 3.8223 | |
| D | 0.507 | 0.4495 | 8 | 3.5961 | Average 3.622 |
| E | 0.659 | 0.5802 | 4 | 2.3207 | STDEV 0.188 |
| F | 0.843 | 0.7379 | 2 | 1.4759 | %RSD 5.20 |
| G | 0.913 | 0.7981 | 1 | 0.7981 | Horrat 0.24 |

| T-Ctrl-D1-12f-b | | | | | |
|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | |
| A | 0.047 | 0.0535 | 64 | 3.425 | |
| B | 0.050 | 0.0563 | 32 | 1.803 | |
| C | 0.090 | 0.0904 | 16 | 1.446 | |
| D | 0.219 | 0.2016 | 8 | 1.613 | Average 1.591 |
| E | 0.422 | 0.3758 | 4 | 1.503 | STDEV 0.157 |
| F | 0.708 | 0.6225 | 2 | 1.245 | %RSD 9.89 |
| G | 0.832 | 0.7289 | 1 | 0.729 | Horrat 0.46 |

| T-F2-D1-12f-b | | | | | |
|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | |
| A | 0.081 | 0.0830 | 64 | 5.3148 | |
| B | 0.143 | 0.1364 | 32 | 4.3661 | |
| C | 0.318 | 0.2864 | 16 | 4.5824 | |
| D | 0.524 | 0.4640 | 8 | 3.7123 | Average 4.220 |
| E | 0.703 | 0.6174 | 4 | 2.4698 | STDEV 0.453 |
| F | 0.844 | 0.7387 | 2 | 1.4774 | %RSD 10.73 |
| G | 0.923 | 0.8068 | 1 | 0.8068 | Horrat 0.50 |

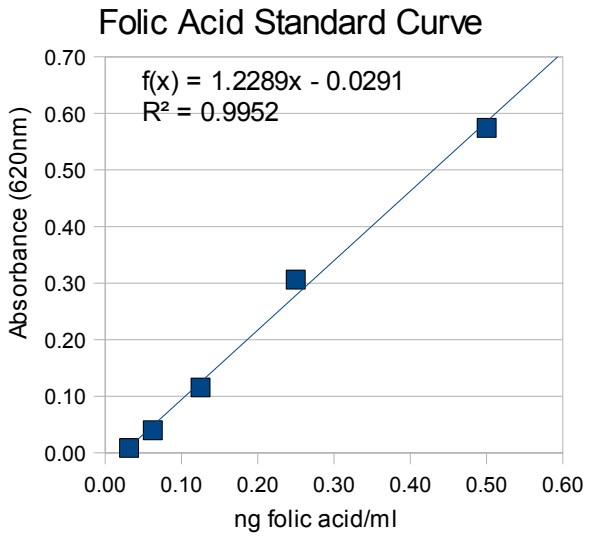
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-5 Plate 1 | T-Ctrl-D2-12f-a | 1.0217 | 2 | 1.336 | 0.1336 | 1 | 0.1307 | 44.62 | 0.236 | n/a | n/a |
| | T-F2-D2-12f-b | 1.0180 | | 3.193 | 0.3193 | 2.5 | 0.7841 | 43.12 | 1.379 | 3.530 | 39.05 |
| | T-F2-D2-12f-a | 1.0215 | | 3.288 | 0.3288 | 2.5 | 0.8047 | 43.86 | 1.433 | 3.530 | 40.61 |
| | T-F1-D2-12f-b | 1.0373 | | 4.105 | 0.4105 | 2.5 | 0.9894 | 42.91 | 1.733 | 3.510 | 49.38 |
| | T-Ctrl-D2-12f-b | 1.0313 | | 1.529 | 0.1529 | 1 | 0.1482 | 40.54 | 0.249 | n/a | n/a |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.008 | 0.009 | 0.004 | 0.011 | 0.036 | 0.039 | 0.044 | 0.043 | 0.062 | 0.064 | 0.032 | 0.033 |
| B | 0.038 | 0.042 | 0.017 | 0.025 | 0.069 | 0.091 | 0.088 | 0.080 | 0.118 | 0.125 | 0.036 | 0.037 |
| C | 0.103 | 0.128 | 0.068 | 0.062 | 0.217 | 0.194 | 0.232 | 0.191 | 0.271 | 0.311 | 0.066 | 0.072 |
| D | 0.315 | 0.298 | 0.182 | 0.179 | 0.479 | 0.513 | 0.493 | 0.462 | 0.640 | 0.602 | 0.175 | 0.204 |
| E | 0.642 | 0.507 | 0.403 | 0.422 | 0.735 | 0.783 | 0.719 | 0.689 | 0.788 | 0.774 | 0.468 | 0.520 |
| F | 0.838 | 0.760 | 0.726 | 0.752 | 0.863 | 0.866 | 0.879 | 0.796 | 0.965 | 0.963 | 0.744 | 0.774 |
| G | 0.899 | 0.909 | 0.972 | 0.928 | 0.987 | 0.985 | 1.015 | 1.005 | 1.021 | 1.039 | 0.923 | 0.960 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | T-Ctrl-D2-12f-a | T-F2-D2-12f-b | T-F2-D2-12f-a | T-F1-D2-12f-b | T-Ctrl-D2-12f-b |
|---|---|---|---|---|---|---|
| A | 0.009 | 0.007 | 0.038 | 0.043 | 0.063 | 0.032 |
| B | 0.040 | 0.021 | 0.080 | 0.084 | 0.122 | 0.037 |
| C | 0.116 | 0.065 | 0.206 | 0.212 | 0.291 | 0.069 |
| D | 0.306 | 0.181 | 0.496 | 0.478 | 0.621 | 0.190 |
| E | 0.574 | 0.412 | 0.759 | 0.704 | 0.781 | 0.494 |
| F | 0.799 | 0.739 | 0.865 | 0.837 | 0.964 | 0.759 |
| G | 0.904 | 0.950 | 0.986 | 1.010 | 1.030 | 0.942 |




| T-Ctrl-D2-12f-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.007 | 0.0296 | 64 | 1.8927 | | |
| B | 0.021 | 0.0411 | 32 | 1.3148 | | |
| C | 0.065 | 0.0767 | 16 | 1.2270 | | |
| D | 0.181 | 0.1706 | 8 | 1.3647 | Average | 1.336 |
| E | 0.412 | 0.3590 | 4 | 1.4361 | STDEV | 0.088 |
| F | 0.739 | 0.6253 | 2 | 1.2505 | %RSD | 6.58 |
| G | 0.950 | 0.7965 | 1 | 0.7965 | Horrat | 0.31 |

| T-F2-D2-12f-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.038 | 0.0542 | 64 | 3.4680 | | |
| B | 0.080 | 0.0885 | 32 | 2.8315 | | |
| C | 0.206 | 0.1909 | 16 | 3.0541 | | |
| D | 0.496 | 0.4273 | 8 | 3.4180 | Average | 3.193 |
| E | 0.759 | 0.6410 | 4 | 2.5640 | STDEV | 0.303 |
| F | 0.865 | 0.7274 | 2 | 1.4548 | %RSD | 9.50 |
| G | 0.986 | 0.8259 | 1 | 0.8259 | Horrat | 0.45 |

| T-F1-D2-12f-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.063 | 0.0750 | 64 | 4.7985 | | |
| B | 0.122 | 0.1225 | 32 | 3.9211 | | |
| C | 0.291 | 0.2602 | 16 | 4.1640 | | |
| D | 0.621 | 0.5289 | 8 | 4.2311 | Average | 4.105 |
| E | 0.781 | 0.6594 | 4 | 2.6376 | STDEV | 0.163 |
| F | 0.964 | 0.8080 | 2 | 1.6159 | %RSD | 3.97 |
| G | 1.030 | 0.8618 | 1 | 0.8618 | Horrat | 0.19 |

| T-F2-D2-12f-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.043 | 0.0590 | 64 | 3.7753 | | |
| B | 0.084 | 0.0921 | 32 | 2.9460 | | |
| C | 0.212 | 0.1958 | 16 | 3.1323 | | |
| D | 0.478 | 0.4123 | 8 | 3.2983 | Average | 3.288 |
| E | 0.704 | 0.5967 | 4 | 2.3866 | STDEV | 0.355 |
| F | 0.837 | 0.7049 | 2 | 1.4098 | %RSD | 10.81 |
| G | 1.010 | 0.8455 | 1 | 0.8455 | Horrat | 0.51 |

| T-Ctrl-D2-12f-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.032 | 0.0499 | 64 | 3.192 | | |
| B | 0.037 | 0.0535 | 32 | 1.712 | | |
| C | 0.069 | 0.0797 | 16 | 1.276 | | |
| D | 0.190 | 0.1780 | 8 | 1.424 | Average | 1.529 |
| E | 0.494 | 0.4259 | 4 | 1.703 | STDEV | 0.215 |
| F | 0.759 | 0.6413 | 2 | 1.283 | %RSD | 14.08 |
| G | 0.942 | 0.7899 | 1 | 0.790 | Horrat | 0.66 |

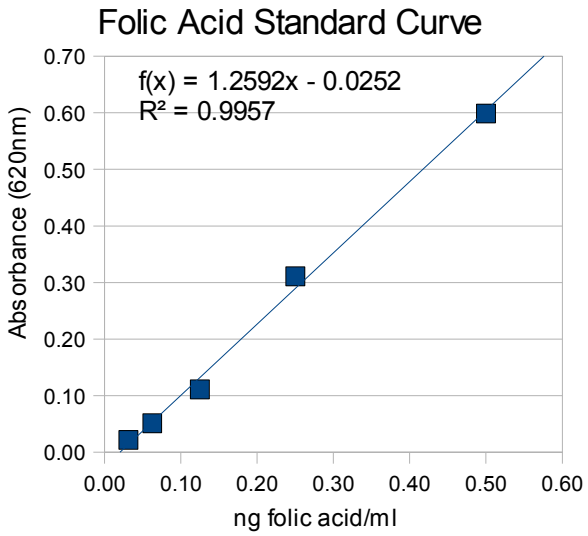
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-5 Plate 2 | T-F1-D2-12f-a | 1.0112 | 2 | 4.111 | 0.4111 | 2.5 | 1.0164 | 45.17 | 1.854 | 3.510 | 52.82 |
| | C-D2-a | 1.0180 | | 3.019 | 0.3019 | 1 | 0.2965 | 12.33 | 0.338 | n/a | n/a |
| | C-D3-a | 1.0166 | | 2.903 | 0.2903 | 1 | 0.2855 | 12.07 | 0.325 | n/a | n/a |
| | N-D1-b | 1.0090 | | 1.376 | 0.1376 | 1 | 0.1364 | 53.99 | 0.296 | n/a | n/a |
| | N-D1-a | 1.0062 | | 1.162 | 0.1162 | 1 | 0.1155 | 52.69 | 0.244 | n/a | n/a |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.025 | 0.018 | 0.050 | 0.053 | 0.037 | 0.039 | 0.040 | 0.041 | 0.035 | 0.038 | 0.029 | 0.041 |
| B | 0.058 | 0.044 | 0.127 | 0.130 | 0.068 | 0.081 | 0.079 | 0.069 | 0.038 | 0.041 | 0.037 | 0.037 |
| C | 0.112 | 0.110 | 0.335 | 0.307 | 0.205 | 0.199 | 0.195 | 0.182 | 0.076 | 0.064 | 0.066 | 0.063 |
| D | 0.314 | 0.307 | 0.661 | 0.623 | 0.498 | 0.536 | 0.450 | 0.455 | 0.194 | 0.210 | 0.152 | 0.141 |
| E | 0.625 | 0.573 | 0.786 | 0.816 | 0.770 | 0.734 | 0.721 | 0.755 | 0.441 | 0.436 | 0.374 | 0.367 |
| F | 0.842 | 0.748 | 1.004 | 0.972 | 0.982 | 0.953 | 1.008 | 0.927 | 0.778 | 0.676 | 0.741 | 0.756 |
| G | 0.951 | 0.951 | 1.079 | 1.065 | 1.080 | 1.081 | 1.092 | 1.097 | 0.946 | 0.979 | 0.923 | 0.933 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | T-F1-D2-12f-a | C-D2-a | C-D3-a | N-D1-b | N-D1-a |
|---|---|---|---|---|---|---|
| A | 0.022 | 0.051 | 0.038 | 0.040 | 0.037 | 0.035 |
| B | 0.051 | 0.129 | 0.075 | 0.074 | 0.039 | 0.037 |
| C | 0.111 | 0.321 | 0.202 | 0.189 | 0.070 | 0.064 |
| D | 0.311 | 0.642 | 0.517 | 0.453 | 0.202 | 0.146 |
| E | 0.599 | 0.801 | 0.752 | 0.738 | 0.439 | 0.370 |
| F | 0.795 | 0.988 | 0.967 | 0.967 | 0.727 | 0.749 |
| G | 0.951 | 1.072 | 1.081 | 1.094 | 0.963 | 0.928 |




| T-F1-D2-12f-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.051 | 0.0608 | 64 | 3.8926 | | |
| B | 0.129 | 0.1222 | 32 | 3.9110 | | |
| C | 0.321 | 0.2750 | 16 | 4.4006 | | |
| D | 0.642 | 0.5300 | 8 | 4.2403 | Average | 4.111 |
| E | 0.801 | 0.6563 | 4 | 2.6253 | STDEV | 0.251 |
| F | 0.988 | 0.8048 | 2 | 1.6096 | %RSD | 6.09 |
| G | 1.072 | 0.8715 | 1 | 0.8715 | Horrat | 0.29 |

| C-D2-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.038 | 0.0501 | 64 | 3.2064 | | |
| B | 0.075 | 0.0792 | 32 | 2.5360 | | |
| C | 0.202 | 0.1805 | 16 | 2.8877 | | |
| D | 0.517 | 0.4307 | 8 | 3.4454 | Average | 3.019 |
| E | 0.752 | 0.6176 | 4 | 2.4705 | STDEV | 0.395 |
| F | 0.967 | 0.7883 | 2 | 1.5766 | %RSD | 13.08 |
| G | 1.081 | 0.8783 | 1 | 0.8783 | Horrat | 0.61 |

| N-D1-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.037 | 0.0491 | 64 | 3.1428 | | |
| B | 0.039 | 0.0513 | 32 | 1.6400 | | |
| C | 0.070 | 0.0755 | 16 | 1.2083 | | |
| D | 0.202 | 0.1807 | 8 | 1.4454 | Average | 1.376 |
| E | 0.439 | 0.3684 | 4 | 1.4736 | STDEV | 0.178 |
| F | 0.727 | 0.5972 | 2 | 1.1945 | %RSD | 12.93 |
| G | 0.963 | 0.7846 | 1 | 0.7846 | Horrat | 0.61 |

| C-D3-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.040 | 0.0521 | 64 | 3.3360 | | |
| B | 0.074 | 0.0787 | 32 | 2.5182 | | |
| C | 0.189 | 0.1699 | 16 | 2.7187 | | |
| D | 0.453 | 0.3798 | 8 | 3.0381 | Average | 2.903 |
| E | 0.738 | 0.6063 | 4 | 2.4253 | STDEV | 0.360 |
| F | 0.967 | 0.7884 | 2 | 1.5768 | %RSD | 12.39 |
| G | 1.094 | 0.8892 | 1 | 0.8892 | Horrat | 0.58 |

| N-D1-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.035 | 0.0479 | 64 | 3.067 | | |
| B | 0.037 | 0.0495 | 32 | 1.584 | | |
| C | 0.064 | 0.0712 | 16 | 1.140 | | |
| D | 0.146 | 0.1364 | 8 | 1.091 | Average | 1.162 |
| E | 0.370 | 0.3142 | 4 | 1.257 | STDEV | 0.222 |
| F | 0.749 | 0.6148 | 2 | 1.230 | %RSD | 19.1 |
| G | 0.928 | 0.7571 | 1 | 0.757 | Horrat | 0.9 |

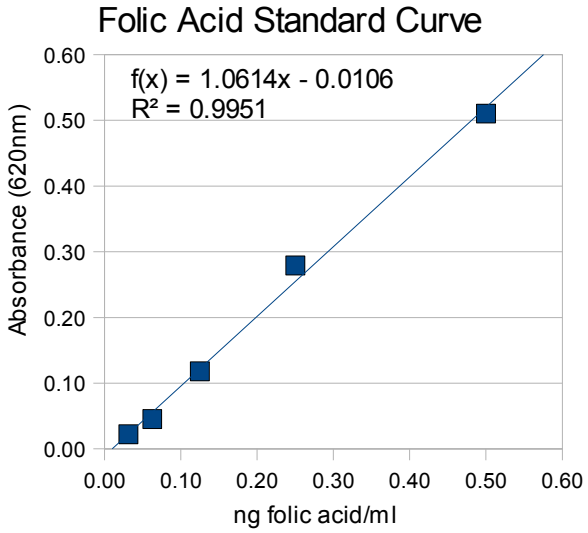
| Test ID | Samples | Sample Size (g) | Conc. of standard curve (ng/ml) | Conc. from plate (ng/ml) | Actual folic acid content (ug) | Additional dilution (x) | Folic acid conc (ug FA/g sample) wwb | % Moisture | Folic acid conc (ug/g) (dwb) | Theoretical folic acid conc (ug/g) | % Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-5 Plate 3 | T-F1-D1-12f-b | 1.0695 | 2 | 6.101 | 0.6101 | 2.5 | 1.4261 | 44.77 | 2.582 | 3.510 | 73.57 |
| | M-F1-D1-1-b | 1.0179 | | 6.044 | 0.6044 | 2.5 | 1.4845 | 54.2 | 3.241 | 3.604 | 89.93 |
| | N-D2-a | 1.0267 | | 1.537 | 0.1537 | 1 | 0.1497 | 51.98 | 0.312 | n/a | n/a |
| | M-Ctrl-D2-0-b | 1.0285 | | 1.245 | 0.1245 | 1 | 0.1210 | 54.69 | 0.267 | n/a | n/a |

Raw Absorbance Data (620nm) After Accounting for Blanks (Row H)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.025 | 0.020 | 0.026 | 0.026 | 0.086 | 0.089 | 0.086 | 0.093 | 0.030 | 0.032 | 0.015 | 0.030 |
| B | 0.048 | 0.043 | 0.047 | 0.041 | 0.187 | 0.189 | 0.180 | 0.199 | 0.043 | 0.030 | 0.033 | 0.027 |
| C | 0.119 | 0.117 | 0.126 | 0.141 | 0.378 | 0.395 | 0.347 | 0.386 | 0.069 | 0.086 | 0.049 | 0.063 |
| D | 0.281 | 0.277 | 0.335 | 0.302 | 0.583 | 0.579 | 0.546 | 0.582 | 0.206 | 0.221 | 0.150 | 0.150 |
| E | 0.529 | 0.492 | 0.590 | 0.603 | 0.661 | 0.761 | 0.700 | 0.724 | 0.401 | 0.490 | 0.383 | 0.409 |
| F | 0.801 | 0.774 | 0.804 | 0.815 | 0.821 | 0.836 | 0.845 | 0.810 | 0.780 | 0.757 | 0.737 | 0.710 |
| G | 0.939 | 0.918 | 0.930 | 0.901 | 0.940 | 0.883 | 0.937 | 0.849 | 0.957 | 0.959 | 0.936 | 0.928 |

Average Absorbance of the Duplicate Columns from above

| | Standard Curve | | T-F1-D1-12f-b | M-F1-D1-1-b | N-D2-a | M-Ctrl-D2-0-b |
|---|---|---|---|---|---|---|
| A | 0.022 | 0.026 | 0.088 | 0.089 | 0.031 | 0.023 |
| B | 0.046 | 0.044 | 0.188 | 0.190 | 0.036 | 0.030 |
| C | 0.118 | 0.134 | 0.386 | 0.366 | 0.078 | 0.056 |
| D | 0.279 | 0.318 | 0.581 | 0.564 | 0.213 | 0.150 |
| E | 0.510 | 0.596 | 0.711 | 0.712 | 0.445 | 0.396 |
| F | 0.788 | 0.810 | 0.829 | 0.828 | 0.769 | 0.723 |
| G | 0.928 | 0.916 | 0.911 | 0.893 | 0.958 | 0.932 |




| T-F1-D1-12f-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.088 | 0.0897 | 64 | 5.7410 | | |
| B | 0.188 | 0.1933 | 32 | 6.1850 | | |
| C | 0.386 | 0.3986 | 16 | 6.3772 | | |
| D | 0.581 | 0.6002 | 8 | 4.8016 | Average | 6.101 |
| E | 0.711 | 0.7343 | 4 | 2.9372 | STDEV | 0.326 |
| F | 0.829 | 0.8560 | 2 | 1.7119 | %RSD | 5.35 |
| G | 0.911 | 0.9415 | 1 | 0.9415 | Horrat | 0.25 |

| M-F1-D1-1-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.089 | 0.0913 | 64 | 5.8436 | | |
| B | 0.190 | 0.1951 | 32 | 6.2429 | | |
| C | 0.366 | 0.3779 | 16 | 6.0463 | | |
| D | 0.564 | 0.5824 | 8 | 4.6589 | Average | 6.044 |
| E | 0.712 | 0.7358 | 4 | 2.9432 | STDEV | 0.200 |
| F | 0.828 | 0.8551 | 2 | 1.7102 | %RSD | 3.30 |
| G | 0.893 | 0.9224 | 1 | 0.9224 | Horrat | 0.15 |

| M-Ctrl-D2-0-b | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.023 | 0.0313 | 64 | 2.001 | | |
| B | 0.030 | 0.0384 | 32 | 1.228 | | |
| C | 0.056 | 0.0630 | 16 | 1.008 | | |
| D | 0.150 | 0.1512 | 8 | 1.209 | Average | 1.245 |
| E | 0.396 | 0.3832 | 4 | 1.533 | STDEV | 0.216 |
| F | 0.723 | 0.6915 | 2 | 1.383 | %RSD | 17.39 |
| G | 0.932 | 0.8882 | 1 | 0.888 | Horrat | 0.82 |

| N-D2-a | | | | | | |
|---|---|---|---|---|---|---|
| Row | Abs at 620nm | Conc. from std. curve (ng/ml) | Dilution | Folate conc. (ng/ml) | | |
| A | 0.031 | 0.0391 | 64 | 2.5021 | | |
| B | 0.036 | 0.0441 | 32 | 1.4109 | | |
| C | 0.078 | 0.0831 | 16 | 1.3298 | | |
| D | 0.213 | 0.2111 | 8 | 1.6884 | Average | 1.537 |
| E | 0.445 | 0.4297 | 4 | 1.7187 | STDEV | 0.196 |
| F | 0.769 | 0.7347 | 2 | 1.4694 | %RSD | 12.73 |
| G | 0.958 | 0.9128 | 1 | 0.9128 | Horrat | 0.6 |

# APPENDIX B

# DETAILED FOLATE ANALYSIS METHOD

# Testing Total Folate in Cereal Products

Revision 10

## Table of Contents

**Notes about the entire analysis – Please read first:**

The entire analysis needs to be done under subdued light. Any sterile filtering needs to be done in the bio-hood in the micro lab. All non-disposable glassware must be burned for 1 hour at 250ºC (475F) prior to use (we often used the product development labs' ovens). Use a separate scoop for measuring out folic acid so no contamination occurs. If there is an * after the material, it needs to be autoclaved prior to use.

**Preparation for the analysis – Inoculum**

Materials needed:

- Lactobacilli broth AOAC (Difco)
- 0.22 um Sterilizing 250mL filter system with PES membrane (VWR *87006-062)*
- 2 sterile needles (20g, 1" works well)
- 2 sterile syringes (1cc)
- Lyophilized L. casei (ATCC 7469)
- Lactobacilli Agar AOAC (Difco)
- 250ml Erlenmeyer flask

If sterile transfers and techniques aren't used in this step, **many week's worth of work will be wasted with contaminated bacteria so do a good job now!!!!!**

1. Weigh 3.8 g of Lactobacilli broth into 100 ml water using a 250ml Erlenmeyer flask. Boil for 2-3 min, then sterile filter using the 0.22 um sterile filter system.
2. Using sterile techniques and the sterile needle and syringe, take 1 ml of media made in step 1 and inject it into the L. casei vial. Mix until homogenous.
3. Transfer out using a fresh~0.15ml solution and dispense into media made in step 1 and cap.
4. Incubate for 24 hours at 37°C. Store in refrigerator until use.
5. Re-transfer using a sterile loop (sterilized by ethanol and flame) into new sterile lactobacilli broth (as prepared in step 1) every 5-7 days to keep the inoculum fresh.

**Preparation for the analysis – Rat Plasma**

Materials needed:

- Glass funnel (of adequate size to fit the filter paper)
- Filter paper of large pore size
- 250 ml Erlenmeyer flask
- 50 ml Erlenmeyer flask
- Single use syringe filter 0.22 um (obtained from Chemstores)
- 10ml sterile syringe
- Sterile screw top vials (2ml)
- Test tubes to fit centrifuge

1. Add 5% acetic acid solution to 250ml flask containing activated charcoal until charcoal is just submersed. Cover flask and mix on mechanical shaker for 1 hour. Drain the charcoal using the funnel and filter paper and rinse with distilled water.

2. Rat serum is mixed with one-tenth weight of acid treated charcoal (if you want 10g rat serum, you mix it with 1g acid treated charcoal) in 50 ml Erlenmeyer flask. Mix flask on ice on the orbital mechanical shaker in the lab for one hour at speed 120.
3. Transfer mixture to a tube suitable for the centrifuge. Centrifuge at ~5500 rpm for sufficient time to retrieve the supernatant.
4. Filter the supernatant using the syringe filter into the sterile screw-top vials.
5. Aliquots can be stored at -50°C.

Sources:

Tamura T. 1998. Determination of food folate. Nutritional Biochemistry 9:285-93.

Phillips KM, Wunderlich KM, Holden JM, Exler J, Gebhart SE, Haytowitz DB, Beecher GR, Doherty RF. 2005. Stability of 5-methyltetrahydrofolate in frozen fresh fruits and vegetables. Food chem. 92:587-95.

Partridge SM. 1949. Displacement Chromatography on Synthetic Ion-exchange Resins. Biochem J. 44:521-7.

**Day one of the analysis – Enzymatic treatment of sample**

Materials needed:

- Working standard solution (1ug/ml) (see Solutions)
- 125 ml Erlenmeyer flask (1 per sample + 1 for standard)
- pH 7.8 phosphate buffer (30 ml per sample + 30 ml for standard) (see Solutions)
- 18x150mm test tubes (1 per sample)
- Graduated cylinder capable of 30 ml
- 1-Octanol (99% pure)
- 50 ml beaker (one per sample)
- Protease solution (See Solutions) (1 ml per sample + 1 for standard)
- α-amylase solution (See Solutions) (1 ml per sample + 1 for standard)

Standard solution (2ng/ml):

1. Accurately pipette 1.0 ml working standard solution (1ug/ml) to 125 ml Erlenmeyer flask containing 20 ml pH 7.8 phosphate buffer; mix; and add 30 ml water.
2. Continue treatment starting with step 4.

Samples:

1. Accurately weigh an amount of sample containing ~1ug folate (about 0.25-1.0g (dry basis) solids) into a 125-Erlenmeyer flask. If sample is low in folate, do not use weigh more than 1.0g; instead on day two, do not do the additional dilution as outlined in step 5 (but still dilute the standard).
2. Add 20 ml pH 7.8 phosphate buffer; mix thoroughly by gently swirling the flask.
3. Add 30 ml distilled water to each flask.
4. Add .5 ml octanol (antifoam) to all flasks.
5. Cover flasks with inverted 50 ml beaker and autoclave 15 min at 121°-123°C and cool in an ice water bath until room temperature.
6. Add an additional 10 ml 7.8 phosphate buffer to each flask. Note, this does not need to be sterile.

7. Add 1 ml protease solution, swirl to mix, wrap flasks in foil (to occlude light), and incubate 3 hours at 37°C.
8. Inactivate enzyme with a boiling water bath for 3 min; cool in an ice water bath.
9. Add 1 ml α-amylase solution to each flask, swirl to mix and rewrap and incubate 2 hours at 37°C.
10. Add 0.1 ml treated rat plasma. Incubate 16 hours at 37°C.

Note: You do have some leeway with the timing of adding the enzymes. The most important part though is you do add them in the order and be sure to deactivate the protease before adding the amylase.

**Day two of the analysis - Plating**

Materials needed:

- Depletion media in screw cap tube (see Solutions)
- HCl (1 part HCl to 1 part $H_20$)
- 100 ml volumetric flask (1 per sample + 1 for standard)
- Whatman 2V filter paper (1 for each sample + 1 for standard)
- Single use syringe filters 0.22 um filters (1 for each sample + 1 for standard)
- Sterile screw top vials (2ml) (1 for each sample + 1 for standard)
- Double strength basal medium (15 ml per plate)
- 0.22 um Sterilizing 250mL filter system with PES membrane (VWR *87006-062)*
- Sterile pipetting troughs
- Sterile microtiter plates – Falcon 1172 96-well non tissue culture plate flat bottomed, sold by VWR
- 150 ul pipette tips (yellow) * (1 box per plate + 1)
- Glass Funnels (1 for each sample + 1 for standard)
- 50 ml beaker
- 2 20ml sterile syringes
- 5 ml volumetric flasks (1 for each sample + 1 for standard)
- Test tubes (OSI tubes work best, 25mm x 200mm) tightly covered with tin foil *

1. Six hours previous to planned plating time, take a loop of bacteria from refrigerated culture (see "Preparation for the Analysis – Inoculum") and transfer it to a depletion media tube. Ensure sterility of transfer by sterilizing loop with ethanol and flame. Place in incubator at 37°C for the six hours.
2. Inactivate enzymes within the samples from the end of day 1 analysis in a boiling water bath for 3 min; cool in an ice water bath.
3. Adjust samples and standard to pH 4.5 with HCl (typically takes about 3/4" of pasteur pipette). Quantitatively transfer to a 100 ml volumetric flask and fill to volume with distilled water.
4. Filter approximately 20 ml of the pH adjusted sample through 2V filter paper into a test tube.
5. Pipette 1 ml of the standard into 5 ml volumetric flask and fill to volume with distilled water. The standard should always be diluted this way. Do an appropriate dilution of samples so that the expected folic acid content falls close to the standard curve's concentration (2ng/ml).

6. Filter sterilize approximately 2 ml of diluted filtered solution (from step 5 and 6) using 0.22 um filter into sterile screw cap vials.
7. Filter sterilize ~20ml water using 0.2 um filter system into a pipetting trough.
8. Using multi-channel pipette, pipette 150 ul of water to wells A1-H12 (all the wells) of a sterile microtiter plate.
9. Pipette 150 ul standard extract to wells G1-G2. For each sample extract, pipette 150 ul in duplicate into wells in row G, i.e., 150 ul of sample 1 extract to each well G3 and G4, 150 ul of sample 2 extract to G5 and G6, etc., up to G12. Therefore you can run five sample extracts and one standard extract per microtiter plate.
10. Mix contents of row G to homogeneity by using a multi-channel pipette and repeating aspiration and delivery steps into same wells.
11. Make serial dilutions (x2) of standard and samples by transferring 150 ul from wells G1-G12 to F1-F12 and mixing, then from F1-F12 to E1-E12 and mixing, etc. For samples A1-A12 at top of plate withdraw 150 ul from each well after mixing and discard.
12. Filter sterilize 20ml media using 0.22 um filter system and sterile syringe.
13. Add 20 ul of incubated depleted bacteria culture (see step 1) to the media previously filtered into the OSI test tube for every 20 ml of media.
14. Vortex mix the test tube for 30 seconds. Pour into pipette trough. Add 150 ul inoculated media to wells A1-H12 (all the wells) (row H becomes the inoculated blank row).
15. Put cover on microtiter plate. Put plate into a sterile bag and incubate 20-24 hours at 37°C.

**Day three of the analysis – Analyzing plate**

Materials needed:

- 150 ul pipette tips *

1. After incubation time has passed, mix contents of each well using a multi-channel pipette with sterile tips. Ensure there are absolutely no air bubbles in any of the wells; this will distort the optical density (absorbance) reading.
2. Read the plate with the microtiter plate reader in Dr. Davidson's lab (see Appendix B-2). Instructions for the machine are in appendix B. Verify that the standard curve has a good linear portion and that a four point regression line fits it well (see Appendix B-3).

## Appendix B-1: Solutions

**4N KOH**
Materials needed:

- 1 L volumetric flask
- Plastic bottle for storage
- Potassium hydroxide

Add 224 g potassium hydroxide in 1 L water.

**4N NaOH**
Materials needed:

- 1 L volumetric flask
- Plastic bottle for storage
- Sodium hydroxide pellets (EMD SX0590-1) Gr ACS

Add 160 g NaOH in 1 L water.

**.1M pH 7.0 phosphate buffer**
Materials needed:

- 1 L volumetric flask
- 4 N KOH
- 600 ml beaker
- Potassium phosphate monobasic, Gr ACS crystals

Dissolve 13.61 g potassium phosphate monobasic in 1 L volumetric flask and dilute with distilled water to 1 L. Pour at least 500 ml into the beaker and adjust the pH to 7.0 with 4N potassium hydroxide.

**Stock Solution (100ug/ml)**
Materials needed:

- 500 ml low actinic volumetric flask
- USP grade folic acid (Sigma F8798-5G)

Accurately weigh 50 mg USP folic acid that has been dried to constant weight (per instructions in official method) and dissolve in .1M, pH 7.0 phosphate buffer in 500 ml low-actinic volumetric flask. Dilute to volume with .1M phosphate buffer. Top with opaque glass stopper. Store in refrigerator for up to 1 week.

**Working standard solution (1ug/ml)**
Materials needed:

- 600 ml beaker
- 500 ml low-actinic volumetric flask
- (2) 50 ml beakers
- HCl (1 part HCl to 1 part H20)

- 4N KOH
- Folic acid stock solution (100ug/ml)

Bring stock solution to room temperature (to accurately pipette). Fill large beaker with ~450 ml of water and add 5 ml stock solution (100ug/ml). Adjust pH to 7.5 with HCl or KOH depending on the starting pH of the solution. Dilute the HCl or KOH with distilled water using the 2 small beakers if necessary (it probably will be because of the very low buffering ability of this standard solution). Quantitatively transfer the adjusted solution to the 500 ml flask and fill to volume with water. Prepare fresh on day of use.

**pH 7.8 phosphate buffer**
Materials needed:

- Sodium phosphate dibasic anhydrous, granular, Gr ACS
- Ascorbic acid (USP Powder) (Fisher A62-500)
- 100 ml volumetric flask
- 4N NaOH
- 250 ml beaker

Fill flask about half full with water. Add 1.42 g sodium phosphate dibasic and 1.0 g ascorbic acid to flask and dilute to volume. Transfer to beaker. Adjust pH to 7.8 with 4N NaOH. Prepare fresh on day of use.

**Protease solution (make just enough for the day's analysis, 1 ml per sample)**
Materials needed:

- Protease – from Streptomyces griseus, Pronase E from Sigma No P-5147
- 50 ml beaker

Pipette 1 ml of water for every sample + standard plus 1 extra ml of water (for easier pipetting) into the beaker. Add .002 g protease for every 1 ml water. Dissolve by swirling until solution is clear (will be colored). Prepare fresh on day of use. For example, for 5 samples, 7 ml of water should be added to the beaker with .014g protease and dissolved.

**α-amylase solution (make just enough for the day's analysis, 1 ml per sample)**
Materials needed:

- α-amylase – from Aspergillus oryzae, Sigma No A-6211
- Appropriate size volumetric flask (1 ml per sample)

Pipette 1 ml of water for every sample + standard plus 1 extra ml of water (for easier pipetting) into the beaker. Add .02 g amylase for every 1 ml water. Dissolve. Prepare fresh on day of use. For example for 5 samples, 7 ml of water should be added to the beaker with .14g protease and dissolved.

**Depletion media**
Materials needed:

- 250 ml erlenmeyer flask

- Lactobacillus broth AOAC (Difco 290110)
- Folic acid casei media (Difco 282210)
- Screw cap tubes capable of holding 10 ml plus room to mix

Add 1.9 g Lactobacillus broth and 4.7 g folic acid casei media to 100 ml water in 250ml Erlenmeyer flask and boil for 2-3 min. After cooling quickly, dispense 10 ml each into screw cap tubes. Cap the tubes but do not screw tightly!!! Autoclave tubes at 121°C for 20 min, then proceed to tightly screw on caps quickly (while hot), and quickly cool to room temp in an ice bath. Store in refrigerator until use.

**Double strength basal medium**
Materials needed:

- Folic acid casei media (Difco 282210)
- Ascorbic acid (USP Powder) (Fisher A62-500)
- Appropriate size beaker (~20-25 ml per plate)

Suspend 9.4 g casei media and 50 mg ascorbic acid for every 100 ml water. Boil for 1 minute and cool in ice water bath. When cooled cover flask with foil and leave in bath or refrigerate until ready to use (will be filter sterilized previous to plating).

## Appendix B-2: Microplate reader

### Davidson Lab Plate Reader Setup (S-277 ESC)

Reader model: FLUOstar OPTIMA (made by BMG Laboratories)
Software version: 1.30 revision 3
Firmware version: 1.14-0
BMG Labtec technical support: 1-877-264-5227

**1)** Log onto "Lab User" – password: "davidson"
**2)** Double-click "FLUOstar OPTIMA" icon on desktop to launch program
**3)** Select "User" and press "Run"
**4)** "Setup" menu
  **a.** "Reader Configuration" – select "Absorbance"
  **b.** "Filters" – select A-620 in excitation column; emission column should read "empty"
  **c.** "Microplates" – select "Falcon 3070 Culture Plate" from list or add new plate (get specs from manufacturer)
**5)** "Test Setup" menu
  **a.** "Test Protocols" – select name of test or create new test name (i.e. name of the project you're doing). If you have already created one and it doesn't appear on the list, restart the program.
  **b.** "Basic Parameters" tab
    **i.** "Positioning Delay" – enter "0.5" seconds
    **ii.** "Flying Mode" leave unchecked
    **iii.** "No. of kinetic windows" – enter "1" – ignore any further kinetic settings
    **iv.** "Filter Setting" – enter "1" for "no. of chromatics"
    **v.** "Excitation filter" – select "A-620" (whenever doing absorbance readings you want to use only excitation)
    **vi.** "Emission filter" – select "empty"
    **vii.** Ignore "Gain" (this is for a manual gain adjustment, you want an automatic gain adjustment which will be described later)
    **viii.** "Well Scanning" – choose pattern of how to read each well. We used "none." See Help File for more information.
  **c.** "Layout" tab
    **i.** Assign each well as "S" (standard), "X" (unknown), or "B" (blank) as illustrated below:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| A | S7 | S7 | X7 | X7 | X14 | X14 | |
| B | S6 | S6 | X6 | X6 | X13 | X13 | |
| C | S5 | S5 | X5 | X5 | X12 | X12 | |
| D | S4 | S4 | X4 | X4 | X11 | X11 | |
| E | S3 | S3 | X3 | X3 | X10 | X10 | |
| F | S2 | S2 | X2 | X2 | X9 | X9 | |
| G | S1 | S1 | X1 | X1 | X8 | X8 | |
| H | B | B | B | B | B | B | etc… |

**d.** "Concentrations/ Volumes/ Shaking" tab

**i.** "Concentration" – enter "0" for "start concentration" and select "factor"

**ii.** "Volume" – enter "0" for "start volume" and select "factor"

**iii.** "Shaking Options" – select "No Shaking" from drop down menu below "Additional Shaking" – ignore all other shaking options

**iv.** Set dilutions of standard in the table to the right as follows:

| | |
|---|---|
| S1 | 1 |
| S2 | 0.5 |
| S3 | 0.25 |
| S4 | 0.125 |
| S5 | 0.0625 |
| S6 | 0.03125 |
| S7 | 0.015625 |

At this step we ignored that the standard in row G gets diluted by 0.5 (aka when 150 µL standard is added to 150 µL buffer).

**v.** "Injection Timing" tab – everything should be grayed out

**Davidson Lab Plate Reading: First Time**

1) FLUOstar OPTIMA Main Program Screen

a. Press "Plate Out" button (farthest left icon button) to open reader

b. Place plate in reader. Well A1 should be in upper left corner when facing plate reader.

c. Press "Plate In" button (next to "Plate Out")

d. Press "Measure" button (stop light icon button)

e. Double-click appropriate test name (created in "Setup" step 2) a.)

f. "Plate ID's" tab – enter a name from this particular test run (e.g. June 5 hr21)

g. "Gain Adjustment" tab

**vi.** "Required value" – set to "80%"

**vii.** "Filter setting" should read:

| # | Excit. | Emiss. | Gain |
|---|---|---|---|
| 1 | A-620 | empty | (ignore what's here) |

If "Filter setting" appears differently than above go back to steps 2) b. v., vi. and make the necessary changes.

**viii.** Press "Gain Adjustment" button at bottom of screen to set the gain adjustment automatically. A number ~51,000 will appear below "Measurement value for 0% absorption (100% transmission)". This automatic gain setting will automatically be used instead of the manual gain setting.

h. "Sample ID's/Dilution Factors" tab

**ix.** "Dilution" column of will be grayed out for cells corresponding to blanks and standards. Sort the table by content via clicking the "Content" button

at the top of the table or by selecting it from the drop-down menu to the right. Leave "Sample ID" column blank.

**x.** Set dilution *factors* for sample unknowns as follows:

| Well | Content | Sample ID | Dilution |
|---|---|---|---|
| G3 | X1 | | 1 |
| G4 | X1 | | 1 |
| F3 | X2 | | 2 |
| F4 | X2 | | 2 |
| E3 | X3 | | 4 |
| E4 | X3 | | 4 |
| D3 | X4 | | 8 |
| D4 | X4 | | 8 |
| C3 | X5 | | 16 |
| C4 | X5 | | 16 |
| B3 | X6 | | 32 |
| B4 | X6 | | 32 |
| A3 | X7 | | 64 |
| A4 | X7 | | 64 |

Repeat four more times (once for each of the five samples)

i. Press "Start Test Run" button at bottom of screen. Reading a plate with the same setting as described herein takes about two minutes.
j. When reading is completed, eject plate with the "Plate Out" button.
k. Exit program and log off computer.

**Davidson Lab Plate Reading: Second Time and Beyond**

After the initial setup, the following steps are all you should have to do to read additional plates.

**1)** Place plate in reader (see "Plate Reading: First Time" steps 1) a-c)
**2)** Press "Measure" button
**3)** Double-click appropriate test name
**4)** "Gain Adjustment" tab – press "Gain Adjustment" button and wait for reading to appear.
**5)** Press "Start Test Run"

**Davidson Lab Data Analysis**

**1)** FLUOstar OPTIMA Main Program Screen
   a. Press "Evaluation Part" button (spreadsheet icon button on main screen)
   b. Double-click test run of interest
   c. To Export raw data:
      i. Press "Raw Data" tab (bottom of screen)
      ii. Press "Order by Columns" toggle button (top of screen)

iii. Copy and paste data to a new Excel file and save file to a flash drive (this computer doesn't have internet access).

**2)** "Standard Curve" tab
   a. Select "4 Parameter Fit" from the drop-down menu below the graph
   b. Press "Reset" button
   c. Press "Optimize Fit" button
   d. Press "Lin" (linear) toggles button for both x and y axis. The y axis is absorbance units. The x axis is concentration of folic acid.

**Analysis of data**

Using Microsoft Excel makes this very easy. Average the absorbance readings on the duplicate columns (1-2, 3-4, 5-6, etc.). Then plot the first column of data (averaged from columns 1 and 2) on the x-axis and plot the standard curves' concentrations (2ng/ml, 1ng/ml, .5, etc) on the y axis. Use only the points that are on the linear portion of the curve. Determine the formula for the linear trendline of the standard curve. Plug in the unknown absorbance values into the formula. Then multiply that number by the appropriate dilution factor (64, 32, 16, etc). Average the values obtained that fit in the standard curve (excluding those that are way too small or too big). Calculate the average, standard deviation, %RSD (STD*100/Average), and the Horrat value (%RSD/21.33333).

## Appendix B-3: Notes about the analysis

Included here are different things I've learned about the analysis from experience as well as through conversations and emails with Dr. Eitenmiller and his graduate student Sungeun Cho.

- The amount of inoculated depletion media that is added to the folic acid free media for plating is very important. Too much and the bacteria will overgrow and it will make it hard to get good data.
- Use of only the linear portion of the standard curve is important. Use the fourth degree polynomial in the microplate reader software to verify that the microbes grew predictably (the more you do it, the more you'll get a feel for it but as a general statement, you want to see a log growth curve as the concentration of the standard gets higher) but don't use it to determine the unknowns' concentrations. It will become less accurate as the curve plateaus.
- The AOAC method says to add a buffer to the wells whereas I started to use water based on Eitenmillers' suggestion. I've gotten way better results using the water then from using buffer.
- Cryoprotection of the bacteria is certainly accepted in many articles in the literature. However, I have gotten better results using the weekly transferred bacteria. Eitenmiller's lab never cryoprotects the bacteria.
- You should store the filtered extract in their sterile screw cap vials for 1 week in the refrigerator in case you have to re-run the samples due to your dilution being off. You should, however, analyze right away or 1 day after the extraction to ensure no folate loss during storage. This is based off of Sungeun Cho's advice.
- As long as the bacteria are transferred in a sterile manner every 5-7 days, you should be able to use the same bacteria for an unlimited period of time.
- If a plate has contamination throughout the place (as determined by significant growth in the inoculated blank row as well as high growth in the low concentration rows), then it is best to throw out all your bacteria and start fresh.
- The incubation time for the plates can change. I always test the plate after 20 hours of incubation because that is usually the time the plate is done. However due to many factors, the plates can take longer. You can tell when a plate is done by looking at the 4-point regression curve of the standard that is given by the computer. If it starts to plateau after the fifth point, the plate is most likely done. Experience will lead to a better understanding of this. If a plate is not done incubating (as determined after analyzing the plate), recover and place back in the incubator for additional time.

# APPENDIX C

# STATISTICAL ANALYSIS OF GUADALAJARA STUDY

Statistics on the analysis of the masa and the tortillas for both the concentration and percent retention are included. Please note that the statistics used for Table III come from the percent retention even though the table shows concentration. This was done because the percent retention gives a more accurate picture of the true loss of folic acid between the samples since it has the theoretical values taken into account. Highlighted values indicate information of interest. Below is the SAS code used. The data was originally pulled from an excel file; however, all the data is in Appendix A.

```
data content;set content;
concentration=coated;coating='Coated';output;
concentration=uncoated;coating='Uncoated';output;
concentration=control;coating='Control';output;
run;
data loss;set loss;
percent=coated;coating='Coated';output;
percent=uncoated;coating='Uncoated';output;
run;

data content1;set content;
if time^=.;
data content2;set content;
if type^='a';
data loss1;set loss;
if time^=.;
data loss2;set loss;
if type^='a';
run;
title2 'Analysis of Masa';
proc mixed data=content1;
class time day coating;
model concentration=day time coating;
lsmeans day;
lsmeans time coating/pdiff adjust=tukey;
run;
proc mixed data=loss1;
class time day coating;
model percent=day time coating;
lsmeans day  coating;
lsmeans time/pdiff adjust=tukey;
run;

title2 'Analysis of Tortilla';
proc mixed data=content2;
class type day coating;
model concentration=day type coating;
lsmeans day type;
lsmeans coating/pdiff adjust=tukey;
run;
proc mixed data=loss2;
class type day coating;
model percent=day type coating;
lsmeans day type coating;
run;
```

The SAS System
Analysis of Masa
11:22 Wednesday, March 18, 2009

The Mixed Procedure

Model Information

| | |
|---|---|
| Data Set | WORK.CONTENT1 |
| Dependent Variable | concentration |
| Covariance Structure | Diagonal |
| Estimation Method | REML |
| Residual Variance Method | Profile |
| Fixed Effects SE Method | Model-Based |
| Degrees of Freedom Method | Residual |

Class Level Information

| Class | Levels | Values |
|---|---|---|
| time | 4 | 0.5 1 2 4 |
| day | 2 | 1 2 |
| coating | 3 | Coated Contro Uncoat |

Dimensions

| | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 10 |
| Columns in Z | 0 |
| Subjects | 1 |
| Max Obs Per Subject | 48 |

Number of Observations

| | |
|---|---|
| Number of Observations Read | 48 |
| Number of Observations Used | 47 |
| Number of Observations Not Used | 1 |

Covariance Parameter Estimates

| Cov Parm | Estimate |
|---|---|
| Residual | 0.1549 |

Fit Statistics

| | |
|---|---|
| -2 Res Log Likelihood | 55.6 |
| AIC (smaller is better) | 57.6 |

The SAS System
Analysis of Masa
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The Mixed Procedure

Fit Statistics

| | |
|---|---|
| AICC (smaller is better) | 57.7 |
| BIC (smaller is better) | 59.3 |

Type 3 Tests of Fixed Effects

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| day | 1 | 40 | 40.32 | <.0001 |
| time | 3 | 40 | 5.07 | 0.0045 |
| coating | 2 | 40 | 77.02 | <.0001 |

Least Squares Means

| Effect | coating | time | day | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|---|---|
| day | | | 1 | 1.5757 | 0.08226 | 40 | 19.15 | <.0001 |
| day | | | 2 | 0.8456 | 0.08033 | 40 | 10.53 | <.0001 |
| time | | 0.5 | | 1.4960 | 0.1136 | 40 | 13.17 | <.0001 |
| time | | 1 | | 1.2634 | 0.1190 | 40 | 10.62 | <.0001 |
| time | | 2 | | 1.2078 | 0.1136 | 40 | 10.63 | <.0001 |
| time | | 4 | | 0.8754 | 0.1136 | 40 | 7.71 | <.0001 |
| coating | Coated | | | 1.9486 | 0.1019 | 40 | 19.12 | <.0001 |
| coating | Contro | | | 0.2450 | 0.09838 | 40 | 2.49 | 0.0170 |
| coating | Uncoat | | | 1.4384 | 0.09838 | 40 | 14.62 | <.0001 |

Differences of Least Squares Means

| Effect | coating | time | day | _coating | time | day | Estimate | Standard Error | DF |
|---|---|---|---|---|---|---|---|---|---|
| time | | 0.5 | | | 1 | | 0.2326 | 0.1645 | 40 |
| time | | 0.5 | | | 2 | | 0.2883 | 0.1607 | 40 |
| time | | 0.5 | | | 4 | | 0.6206 | 0.1607 | 40 |
| time | | 1 | | | 2 | | 0.05569 | 0.1645 | 40 |
| time | | 1 | | | 4 | | 0.3880 | 0.1645 | 40 |
| time | | 2 | | | 4 | | 0.3323 | 0.1607 | 40 |
| coating | Coated | | | Contro | | | 1.7036 | 0.1417 | 40 |
| coating | Coated | | | Uncoat | | | 0.5102 | 0.1417 | 40 |
| coating | Contro | | | Uncoat | | | -1.1934 | 0.1391 | 40 |

The SAS System
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The Mixed Procedure

Differences of Least Squares Means

| Effect | coating | time | day | _coating | time | day | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|---|---|
| time | | 0.5 | | | 1 | | 1.41 | 0.1652 |
| time | | 0.5 | | | 2 | | 1.79 | 0.0803 |
| time | | 0.5 | | | 4 | | 3.86 | 0.0004 |
| time | | 1 | | | 2 | | 0.34 | 0.7368 |
| time | | 1 | | | 4 | | 2.36 | 0.0233 |
| time | | 2 | | | 4 | | 2.07 | 0.0451 |
| coating | Coated | | | Contro | | | 12.03 | <.0001 |
| coating | Coated | | | Uncoat | | | 3.60 | 0.0009 |
| coating | Contro | | | Uncoat | | | -8.58 | <.0001 |

Differences of Least Squares Means

| Effect | coating | time | day | _coating | time | day | Adjustment | Adj P |
|---|---|---|---|---|---|---|---|---|
| time | | 0.5 | | | 1 | | Tukey-Kramer | 0.4985 |
| time | | 0.5 | | | 2 | | Tukey-Kramer | 0.2910 |
| time | | 0.5 | | | 4 | | Tukey-Kramer | 0.0022 |
| time | | 1 | | | 2 | | Tukey-Kramer | 0.9864 |
| time | | 1 | | | 4 | | Tukey-Kramer | 0.1020 |
| time | | 2 | | | 4 | | Tukey-Kramer | 0.1809 |
| coating | Coated | | | Contro | | | Tukey-Kramer | <.0001 |
| coating | Coated | | | Uncoat | | | Tukey-Kramer | 0.0024 |
| coating | Contro | | | Uncoat | | | Tukey-Kramer | <.0001 |

The SAS System
Analysis of Masa
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The Mixed Procedure

Model Information

| | |
|---|---|
| Data Set | WORK.LOSS1 |
| Dependent Variable | percent |
| Covariance Structure | Diagonal |
| Estimation Method | REML |
| Residual Variance Method | Profile |
| Fixed Effects SE Method | Model-Based |
| Degrees of Freedom Method | Residual |

Class Level Information

| Class | Levels | Values |
|---|---|---|
| time | 4 | 0.5 1 2 4 |
| day | 2 | 1 2 |
| coating | 2 | Coated Uncoat |

Dimensions

| | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 9 |
| Columns in Z | 0 |
| Subjects | 1 |
| Max Obs Per Subject | 32 |

Number of Observations

| | |
|---|---|
| Number of Observations Read | 32 |
| Number of Observations Used | 31 |
| Number of Observations Not Used | 1 |

Covariance Parameter Estimates

| Cov Parm | Estimate |
|---|---|
| Residual | 56.6272 |

Fit Statistics

| | |
|---|---|
| -2 Res Log Likelihood | 184.1 |
| AIC (smaller is better) | 186.1 |

The SAS System
Analysis of Masa
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The Mixed Procedure

Fit Statistics

| | |
|---|---|
| AICC (smaller is better) | 186.3 |
| BIC (smaller is better) | 187.3 |

Type 3 Tests of Fixed Effects

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| day | 1 | 25 | 124.71 | <.0001 |
| time | 3 | 25 | 15.58 | <.0001 |
| coating | 1 | 25 | 25.23 | <.0001 |

Least Squares Means

| Effect | coating | time | day | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|---|---|
| day | | | 1 | 37.0440 | 1.9523 | 25 | 18.97 | <.0001 |
| day | | | 2 | 67.3209 | 1.8813 | 25 | 35.78 | <.0001 |
| coating | Coated | | | 45.3729 | 1.9523 | 25 | 23.24 | <.0001 |
| coating | Uncoat | | | 58.9921 | 1.8813 | 25 | 31.36 | <.0001 |
| time | | 0.5 | | 40.9208 | 2.6605 | 25 | 15.38 | <.0001 |
| time | | 1 | | 49.1567 | 2.8579 | 25 | 17.20 | <.0001 |
| time | | 2 | | 52.4656 | 2.6605 | 25 | 19.72 | <.0001 |
| time | | 4 | | 66.1867 | 2.6605 | 25 | 24.88 | <.0001 |

Differences of Least Squares Means

| Effect | coating | time | day | _coating | time | day | Estimate | Standard Error | DF |
|---|---|---|---|---|---|---|---|---|---|
| time | | 0.5 | | | 1 | | -8.2359 | 3.9046 | 25 |
| time | | 0.5 | | | 2 | | -11.5448 | 3.7626 | 25 |
| time | | 0.5 | | | 4 | | -25.2659 | 3.7626 | 25 |
| time | | 1 | | | 2 | | -3.3089 | 3.9046 | 25 |
| time | | 1 | | | 4 | | -17.0300 | 3.9046 | 25 |
| time | | 2 | | | 4 | | -13.7211 | 3.7626 | 25 |

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The Mixed Procedure

Differences of Least Squares Means

| Effect | coating | time | day | _coating | time | day | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|---|---|
| time | | 0.5 | | | 1 | | -2.11 | 0.0451 |
| time | | 0.5 | | | 2 | | -3.07 | 0.0051 |
| time | | 0.5 | | | 4 | | -6.72 | <.0001 |
| time | | 1 | | | 2 | | -0.85 | 0.4048 |
| time | | 1 | | | 4 | | -4.36 | 0.0002 |
| time | | 2 | | | 4 | | -3.65 | 0.0012 |

Differences of Least Squares Means

| Effect | coating | time | day | _coating | time | day | Adjustment | Adj P |
|---|---|---|---|---|---|---|---|---|
| time | | 0.5 | | | 1 | | Tukey-Kramer | 0.1777 |
| time | | 0.5 | | | 2 | | Tukey-Kramer | 0.0247 |
| time | | 0.5 | | | 4 | | Tukey-Kramer | <.0001 |
| time | | 1 | | | 2 | | Tukey-Kramer | 0.8313 |
| time | | 1 | | | 4 | | Tukey-Kramer | 0.0011 |
| time | | 2 | | | 4 | | Tukey-Kramer | 0.0063 |

The SAS System
Analysis of Tortilla
11:22 Wednesday, March 18, 2009

The Mixed Procedure

Model Information

| | |
|---|---|
| Data Set | WORK.CONTENT2 |
| Dependent Variable | concentration |
| Covariance Structure | Diagonal |
| Estimation Method | REML |
| Residual Variance Method | Profile |
| Fixed Effects SE Method | Model-Based |
| Degrees of Freedom Method | Residual |

Class Level Information

| Class | Levels | Values |
|---|---|---|
| type | 4 | prebake tortilla tortilla12a tortilla12f |
| day | 2 | 1 2 |
| coating | 3 | Coated Contro Uncoat |

Dimensions

| | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 10 |
| Columns in Z | 0 |
| Subjects | 1 |
| Max Obs Per Subject | 48 |

Number of Observations

| | |
|---|---|
| Number of Observations Read | 48 |
| Number of Observations Used | 47 |
| Number of Observations Not Used | 1 |

Covariance Parameter Estimates

| Cov Parm | Estimate |
|---|---|
| Residual | 0.04060 |

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Fit Statistics

| | |
|---|---|
| -2 Res Log Likelihood | 2.1 |
| AIC (smaller is better) | 4.1 |
| AICC (smaller is better) | 4.2 |
| BIC (smaller is better) | 5.8 |

Type 3 Tests of Fixed Effects

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| day | 1 | 40 | 30.74 | <.0001 |
| type | 3 | 40 | 0.56 | 0.6458 |
| coating | 2 | 40 | 310.18 | <.0001 |

Least Squares Means

| Effect | type | coating | day | Estimate | Standard Error | DF | t Value |
|---|---|---|---|---|---|---|---|
| day | | | 1 | 1.4192 | 0.04212 | 40 | 33.70 |
| day | | | 2 | 1.0929 | 0.04113 | 40 | 26.57 |
| type | prebake | | | 1.2564 | 0.05816 | 40 | 21.60 |
| type | tortilla | | | 1.2787 | 0.05816 | 40 | 21.98 |
| type | tortilla12a | | | 1.1944 | 0.05816 | 40 | 20.54 |
| type | tortilla12f | | | 1.2946 | 0.06094 | 40 | 21.25 |
| coating | | Coated | | 1.9772 | 0.05218 | 40 | 37.89 |
| coating | | Contro | | 0.2512 | 0.05037 | 40 | 4.99 |
| coating | | Uncoat | | 1.5397 | 0.05037 | 40 | 30.57 |

Least Squares Means

| Effect | type | coating | day | Pr > \|t\| |
|---|---|---|---|---|
| day | | | 1 | <.0001 |
| day | | | 2 | <.0001 |
| type | prebake | | | <.0001 |
| type | tortilla | | | <.0001 |
| type | tortilla12a | | | <.0001 |
| type | tortilla12f | | | <.0001 |
| coating | | Coated | | <.0001 |
| coating | | Contro | | <.0001 |
| coating | | Uncoat | | <.0001 |

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Differences of Least Squares Means

| Effect | type | coating | day | type | _coating | day | Estimate |
|---|---|---|---|---|---|---|---|
| coating | | Coated | | | Contro | | 1.7261 |
| coating | | Coated | | | Uncoat | | 0.4375 |
| coating | | Contro | | | Uncoat | | -1.2885 |

Differences of Least Squares Means

| Effect | type | coating | day | type | _coating | day | Standard Error | DF |
|---|---|---|---|---|---|---|---|---|
| coating | | Coated | | | Contro | | 0.07253 | 40 |
| coating | | Coated | | | Uncoat | | 0.07253 | 40 |
| coating | | Contro | | | Uncoat | | 0.07124 | 40 |

Differences of Least Squares Means

| Effect | type | coating | day | type | _coating | day | t Value |
|---|---|---|---|---|---|---|---|
| coating | | Coated | | | Contro | | 23.80 |
| coating | | Coated | | | Uncoat | | 6.03 |
| coating | | Contro | | | Uncoat | | -18.09 |

Differences of Least Squares Means

| Effect | type | coating | day | type | _coating | day | Pr > \|t\| |
|---|---|---|---|---|---|---|---|
| coating | | Coated | | | Contro | | <.0001 |
| coating | | Coated | | | Uncoat | | <.0001 |
| coating | | Contro | | | Uncoat | | <.0001 |

Differences of Least Squares Means

| Effect | type | coating | day | type | _coating | day | Adjustment |
|---|---|---|---|---|---|---|---|
| coating | | Coated | | | Contro | | Tukey-Kramer |
| coating | | Coated | | | Uncoat | | Tukey-Kramer |
| coating | | Contro | | | Uncoat | bb | Tukey-Kramer |

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Differences of Least Squares Means

| Effect | type | coating | day | type | _coating | day | Adj P |
|---|---|---|---|---|---|---|---|
| coating | | Coated | | | Contro | | <.0001 |
| coating | | Coated | | | Uncoat | | <.0001 |
| coating | | Contro | | | Uncoat | | <.0001 |

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Model Information

| | |
|---|---|
| Data Set | WORK.LOSS2 |
| Dependent Variable | percent |
| Covariance Structure | Diagonal |
| Estimation Method | REML |
| Residual Variance Method | Profile |
| Fixed Effects SE Method | Model-Based |
| Degrees of Freedom Method | Residual |

Class Level Information

| Class | Levels | Values |
|---|---|---|
| type | 4 | prebake tortilla tortilla12a tortilla12f |
| day | 2 | 1 2 |
| coating | 2 | Coated Uncoat |

Dimensions

| | |
|---|---|
| Covariance Parameters | 1 |
| Columns in X | 9 |
| Columns in Z | 0 |
| Subjects | 1 |
| Max Obs Per Subject | 32 |

Number of Observations

| | |
|---|---|
| Number of Observations Read | 32 |
| Number of Observations Used | 31 |
| Number of Observations Not Used | 1 |

Covariance Parameter Estimates

| Cov Parm | Estimate |
|---|---|
| Residual | 32.5334 |

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Fit Statistics

| | |
|---|---|
| -2 Res Log Likelihood | 170.3 |
| AIC (smaller is better) | 172.3 |
| AICC (smaller is better) | 172.4 |
| BIC (smaller is better) | 173.5 |

Type 3 Tests of Fixed Effects

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| day | 1 | 25 | 42.14 | <.0001 |
| type | 3 | 25 | 0.65 | 0.5913 |
| coating | 1 | 25 | 35.48 | <.0001 |

Least Squares Means

| Effect | type | coating | day | Estimate | Standard Error | DF | t Value |
|---|---|---|---|---|---|---|---|
| day | | | 1 | 43.5953 | 1.4798 | 25 | 29.46 |
| day | | | 2 | 56.9360 | 1.4260 | 25 | 39.93 |
| type | prebake | | | 50.5810 | 2.0166 | 25 | 25.08 |
| type | tortilla | | | 49.9168 | 2.0166 | 25 | 24.75 |
| type | tortilla12a | | | 52.3134 | 2.0166 | 25 | 25.94 |
| type | tortilla12f | | | 48.2514 | 2.1662 | 25 | 22.27 |
| coating | | Coated | | 44.1455 | 1.4798 | 25 | 29.83 |
| coating | | Uncoat | | 56.3859 | 1.4260 | 25 | 39.54 |

Least Squares Means

| Effect | type | coating | day | Pr > \|t\| |
|---|---|---|---|---|
| day | | | 1 | <.0001 |
| day | | | 2 | <.0001 |
| type | prebake | | | <.0001 |
| type | tortilla | | | <.0001 |
| type | tortilla12a | | | <.0001 |
| type | tortilla12f | | | <.0001 |
| coating | | Coated | | <.0001 |
| coating | | Uncoat | | <.0001 |

# APPENDIX D

# DETAILED SAMPLING METHODOLOGY

For all grinding steps, shake vertically the coffee grinder periodically as well tilt the grinder while grinding. Times are somewhat flexible—the important thing is to be consistent with all the same kind of samples.

**Corn:** Take a random 100g sample from each day. Triplicate.

Method to analyze: Let the sample sit out for 10 minutes. Take out 15g. Grind in coffee mill for 1.5 minutes. Take roughly 2-3 g for moisture analysis and 1 gram for folic acid analysis.

**Nixtamal:** Take a random 100g sample from each day. Triplicate.

Method to analyze: Let the sample sit out for 41 minutes. Take out 15g. Grind in coffee mill for 45 seconds (about halfway through, stop and break up clumps that form because the nixtamal is somewhat sticky). Freeze all of the sample for 10 minutes in a plastic weigh boat, stirring halfway to prevent overfreezing. Take roughly 10 g for moisture analysis and 1 gram for folic acid analysis.

**Masa:** Take 100g samples each day that have been stored at 0, 0.5, 1, 2, and 4 hours. At the mill, immediately remove masa (after its designated storage time) and place in sample bag. Sample by dumping out the bucket of masa, cut in half, and scoop out a portion, including all depths of the masa—outside to the center—to account for temperature effects that may occur. Flatten. Place on ice. Triplicate.

Method to analyze: Let the sample sit out for 23 minutes. Take out 15g. Grind in coffee mill for 1 minute (about halfway through, stop and break up clumps that form because the masa is somewhat sticky). Freeze all of the sample for 10 minutes in a plastic weigh boat, stirring halfway through to prevent overfreezing. Take roughly 10 g for moisture analysis and 1 gram for folic acid analysis.

**Tortillas:** Take 100g samples (~3-4 tortillas) from masa that has been held for 0.5 hours each day that have been held at 0, 1, 4, and 12 hours that have been held in the fridge as well as 0, 1, 4, and 12 hours that have been stored at ambient temperature. Triplicate.

Method to analyze: Don't let tortillas thaw (do the following steps with sample directly from freezer). Take out 15g. Grind in coffee mill for 2 minutes. Freeze all of the sample for 10 minutes in a plastic weigh boat, stirring halfway through to prevent overfreezing. Take roughly 10 g for moisture analysis and 1 gram for folic acid analysis.

**Premixes:** Take 5g samples from dosifier each day at the startup and at the stop. Duplicate.

Method to analyze: Let the sample sit out for 5 minutes. Mix ~0.4g of the premix with 250g maltodextrin in the jar mill for 30 minutes with side to side shaking every 7-8 minutes. Using composite sampling, take out 1 gram for analysis.

# APPENDIX E

DATA FROM SCREENING STUDY

$2^2$ x $2^{3-1}$ Split Plot Fractional Factorial Design Used in the Fortified Corn Masa Flour Screening Study

| Run | Iron Source[a] | Iron Level (mg/kg)[b] | Temperature (°C) | Time (min)[c] | pH | %Retention |
|---|---|---|---|---|---|---|
| 1 | E | 40 | 25 | 30 | 9 | 115 |
| 2 | E | 40 | 25 | 120 | 5 | 87 |
| 3 | E | 40 | 35 | 30 | 5 | 92 |
| 4 | E | 40 | 35 | 120 | 9 | 89 |
| 5 | F | 40 | 25 | 30 | 5 | 93 |
| 6 | F | 40 | 25 | 120 | 9 | 96 |
| 7 | F | 40 | 35 | 30 | 9 | 84 |
| 8 | F | 40 | 35 | 120 | 5 | 104 |
| 9 | E | 80 | 25 | 30 | 5 | 86 |
| 10 | E | 80 | 25 | 120 | 9 | 98 |
| 11 | E | 80 | 35 | 30 | 9 | 97 |
| 12 | E | 80 | 35 | 120 | 5 | 101 |
| 13 | F | 80 | 25 | 30 | 9 | 96 |
| 14 | F | 80 | 25 | 120 | 5 | 120 |
| 15 | F | 80 | 35 | 30 | 5 | 103 |
| 16 | F | 80 | 35 | 120 | 9 | 85 |

[a] E = Electrolytic Iron F = Ferrous Fumarate

[b] The levels indicate mg iron per kg masa flour

[c] Masa holding time prior to analysis

As mentioned in the manuscript, no significance was found between any of the data point above. The picture on the next page shows all the data plotted. It was determined that there wasn't any one point that stood out from the rest.

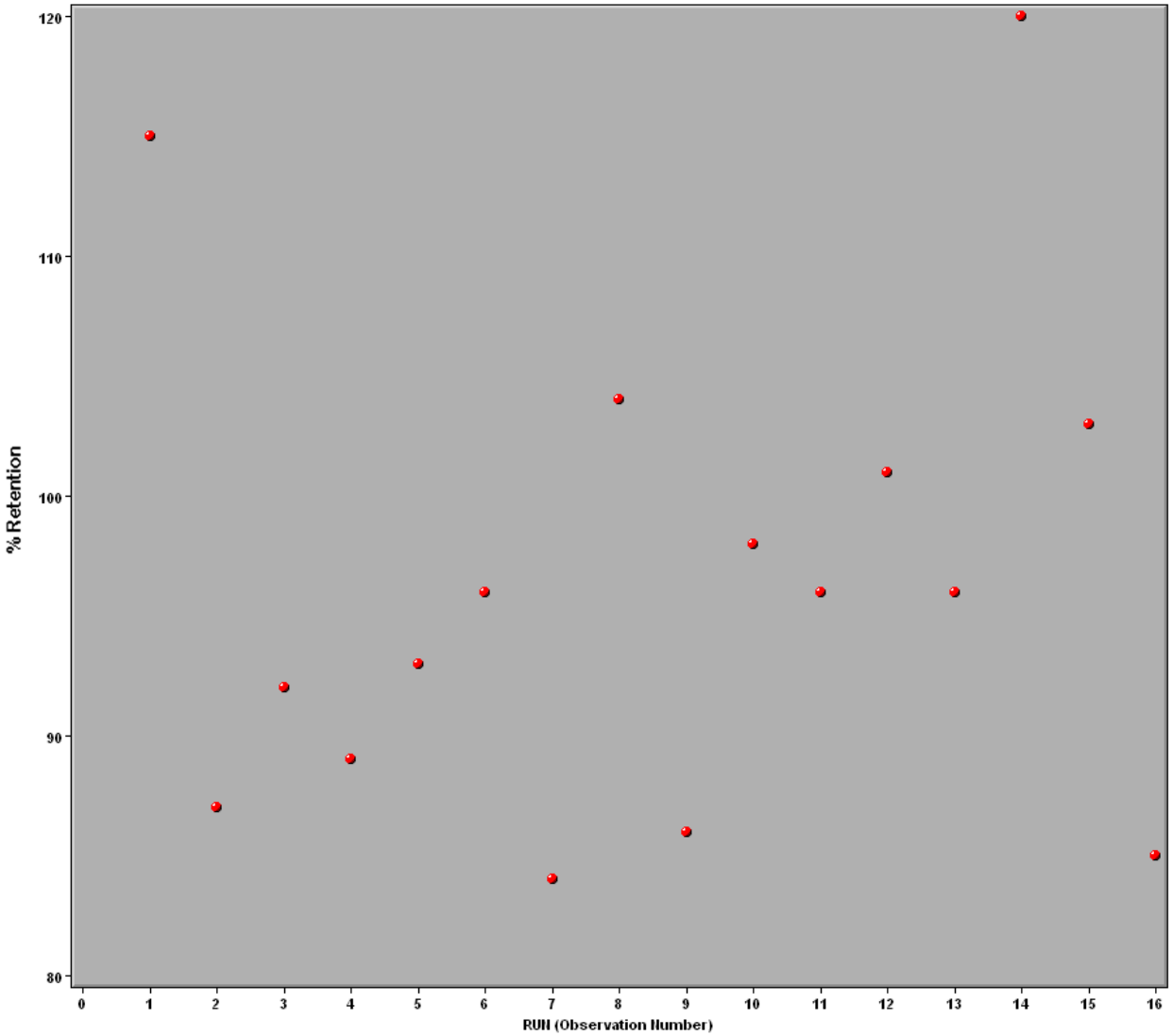


Figure 1 – SAS ADX output from screening study. It was concluded that there weren't any significant outliers and therefore there wasn't any significant variables in the study.