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A new approach for determination of folic acid at trace levels: Using Fe(III)-Folic acid complex to amplify analytical signal

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Abstract

A fast, efficient, and cost-effective, and environmental friendly analytical methodology was developed for preconcentration and determination of trace folic acid in food samples prior to high performance liquid chromatography with diode array detection (HPLC–DAD). The method is based on consist of stable complexes between folic acid and Fe(III) ions at pH 8.0. Then, the formed complexes were extracted to nonionic surfactant phase of PONPE 7.5. Surfactant rich phase (SRP) was separated by decantation and diluted with 300 μL of mixture of 1 M HCl and methanol at 1:1 ratio. The parameters and variables that affected the method were also investigated and optimized in detail. The limits of detection (LOD) of folic acid was 6.06 ng mL^{-1}, the linear range of quantitation for folic acid was 20–1200 ng mL^{-1} and the correlation coefficients of the calibration curves were 0.9976. The average recoveries and relative standard deviations in the analysis of real samples were in the range of 95.1–105.1 % and 1.73–5.25. After validation of method was carried out, then, method was applied to the determination of folic acid in real samples including baby foods, vegetables, cereals, and pharmaceutical samples.

Keywords: Folic Acid, HPLC, cloud point extraction, food samples

1. Introduction

Vitamins are biologically active organic compounds and essential micronutrients for metabolic and physiological functions in the human body. They are necessary for normal health and growth and in sufficient amounts should be supplied by food.[1] Micronutrient deficiency is known to be the most important problem of about one third of the world population. It effects directly mental and physical development in the population and lowers the quality of life. Malnutrition and metabolic diseases can lead to a vitamin deficiency, which shows very significant clinical symptoms, while excessive vitamin intake particularly of fat-soluble vitamins, can result in different diseases.[2]

Folic acid (FA) constitutes a bicyclic pteridine linked by a methylene bridge to p-amino benzoic acid, which is joined by peptide linkage to a single molecule of α-glutamic acid. Deficiency of folic acid resulted to anemia, preventing birth-defects, cardiovascular and cerebrovascular diseases, and certain types of cancer, increasing at the possibility of heart attack.[3] New research and clinical studies have shown that the role of FA in human health is far more important than its use as a vitamin and dietary supplement. The goal of FA supplementation is to reduce the risk of heart diseases and the risk of women giving birth to babies with neural tube defects (spina bifida).[4] The normal levels of FA in human blood serum often ranged between 2 and 15 ng mL^{-1} .[5] However, these levels can be altered by various causes such as increased cellular proliferation occurring in association with pregnancy, lactation, haemolytic anaemia, myeloproliferative disorders, and extensive psoriasis.[6]

Nowadays, it attracts great attention correct determination of vitamins from all sources, including foods, biological materials, and dietary supplements. Analysis of FA, however, is not an easy task because of its presence in extremely lower concentration in real samples, its lower stability under acidic conditions, its sensitiveness against light and high temperature. Numerous methods have been used for the determination of FA including electrochemical sensors[7] spectrophotometry[8], flow injection chemiluminesence[9], fluorimetric[10], high-performance liquid chromatography (HPLC) with ECD[11], LC–MS[12], and capillary electrophoresis.[13] The analytical advantages of HPLC than the other analytical techniques are solvent economy, higher efficiency, mass sensitivity, easy coupling with other techniques, and finally small amounts of sample.[14]

In recent decades, the developments of preconcentration methods to be implemented prior to analytical determinations of trace level compounds have been explored in considerable depth. Separation and preconcentration procedures are always considered of great importance in analytical and environmental chemistry. The use of micellar systems such as CPE has attracted considerable attention in the last decade mainly because it is in agreement with the "green chemistry" principles.[15-16] The comprehensive reviews of the theory and applications of surfactant-mediated separation in analytical chemistry are available.[17-18] Although many successful applications have been reported, several workers agree that these complex systems require a great deal of fundamental research.[19-22] Compared with liquid–liquid extraction, solid phase extraction and matrix solid phase dispersion, CPE has large number of advantages. It is simple and provides higher efficiency enrichment and extraction, and it does not require an organic solvent. Furthermore, the utilized surfactants are degradable and protect the activity of targets.[23-24]

Although many HPLC methods are available for FA determination, no pre-concentration based technique has hitherto been reported that can determine the analyte in real samples with high simplicity. The purpose of this work was to develop and validate a new, simple, low cost, and sensitive method for the determination of folic acid (vitamin B9) in real samples. To the best of our knowledge, this study is the first report describing the application of CPE method for the determination of folic acid by HPLC-DAD system.

2. Experimental

2.1.Instrumentation

The chromatographic system used is equipped with a pump model LC20-AD (Shimadzu), a thermostatic oven, CTO-10 AS (Shimadzu), auto sampler, SIL-20Ac (Shimadzu) and detectors: a DAD detector model SPD-M20A (Shimadzu). An LC solution software (Shimadzu) was used to transfer data to the computer. A Inertsil C18 (250 mm×4,6×5 µm) column were used for chromatographic separation.

A pH meter with a glass-calomel electrode (Selecta, Spain) was used to measure the pH values. A thermostatic water bath (Microtest, Turkey) was used to keep constant the temperature. A centrifuge (Hettich, Universal 120, England) was used for complete phase separation.

2.2.Reagents and Standard Solutions

All reagents used were of analytical grade. Ultra-pure water with a resistivity of 18.2 MΩ was used in all experiments provided by ELGA (Flex III, U.K) water purification system. Possible contaminations arising in laboratory were minimized by stringent precautions at all stages of work. Folic acid (Vitamin B9) was purchased from

Sigma (St. Louis, MO, USA), and methanol and isopropyl alcohol were from Merck (Darmstadt, Germany). 1000 mg L^{-1} of Fe(III) were prepared by using iron nitrate salt bought from Merck. The solutions of PONPE 7.5, a poly-oxyethylene glycol mono ether-type surfactant, (Sigma, St. Loius, MO, USA) was prepared by dissolving in water. The ionic surfactant's solutions (3.0×10^{-3} mol L^{-1} of cetyl pyridinium chloride (CPC), cetyl trimethyl ammonium bromide (CTAB), and sodium dodecyl sulphate (SDS)) were prepared by dissolving an appropriate amount of chemicals (Sigma, St. Loius, MO, USA) in water.

2.3.Chromatographic Analysis

Chromatography was performed by using a Shimadzu HPLC system (Tokyo, Japan) equipped with a quaternary pump, a degasser, a column compartment, and a UV detector. Separations were performed on an Inertsil ODS-3 (5 µm, 4.6 mm × 250 mm) column. Methanol and pH: 3 phosphate buffer including 0.001 mol L^{-1} sodium hexane sulfonate were used as the mobile phase, and an isocratic elution was employed. The other chromatographic conditions were as follows: column temperature: 40 □C, flow rate: 1.0 mL min^{-1}, injection volume: 10 µL, detection wavelength: 284 nm.

2.4.The Proposed Procedure

A 10 ml aliquot of sample containing folic acid (in the range of 25 - 1250 ng mL^{-1}) was placed in a screw-cap centrifuge falcon tube. Then 3.0 mL of pH: 8.00 tris buffer, 0.8 mL of 1000 mg L^{-1} Fe(III), 1.2 mL of 5 % (w/v) PONPE 7.5 were added in order and completed to 50 mL with ultra-pure water. This mixture was incubated in a water bath at 45 ^{0}C for 10 min. The efficient phase separation was carried out by centrifugation at 4000 rpm for 5 min. The aqueous phase was removed with a simple

decantation and the surfactant-rich phase was deposited at the bottom of the tube. Then the surfactant- rich phase was diluted with 300 µL of a mixture of 1 M HCl and Methanol and filtrated by 0.45 µm membrane. Finally, the samples were presented into the HPLC system for analysis.

2.5.Preparation of Samples to Analysis

Sample preparation is one of the most important and difficult steps in the vitamin analysis. In most cases, they have to be extracted from the matrix, however, for the analysis of vitamins in additive raw material or soft drinks, a pre-treatment of the sample may not be necessary. It is important to verify that the chosen sample preparation method is suitable for the analysis of the vitamins of interest, because all vitamins are unstable during common sample preparation methods (boiling for deprotonation. alkali- or acid-treatment). In the case of complex samples, such as multivitamin preparations, foods, plant extracts, serum, or urine, making determinations of trace organic molecules like folic acid requires a lot of tedious work that includes pretreatment steps, and the methods may still result in interference or a matrix effect.[25]

A method published by Mirmoghtadaie et al. was used after a few modifications in the preparation of food samples and certified reference materials (CRM) prior to analysis.[26] According to this method; a vitamin tablet including folic acid was ground and dissolved in 0.10 mol L^{-1} NaOH in a 100 mL standard flask. The mixture was filtered after stirring for 15 min. The other food samples were prepared by dispersing in an appropriate amount of 0.10 mol L^{-1} NaOH solution and stirred for 1 hour. The solution was centrifuged at 5000 rpm for 10 min, after then the mixture was filtered by using a 0.45 µm micropore membrane. And, finally the developed CPE-HPLC method

was applied to 10 mL of the prepared samples after neutralization with 0.01 mol L^{-1} of HCl.

3. Results and Discussions

A lot of pre-experiments were performed in order to ensure the transfer of folic acid molecules to surfactant rich-phase. For this purpose, conventional CPE method was applied to samples for direct transfer of folic acid to micelle medium. Unfortunately, the obtained preconcentration factor was so low according to similar methods. According to our estimates, the transfer of vitamin molecules to surfactant rich phase was very low and the obtained signals were weak, too. For overcoming this problem, it has been utilized the properties of complex formation of folic acid with various metallic ion. Folic acid has functional groups to form complex with metal ions. After the CPE was applied by using metal ions [Cu(II), Mn(II), Fe(II), Fe(III), Al(III), Zn(II), and Ag(I)] the contents of folic acid in the surfactant rich phase were analyzed by HPLC. As can be seen in Figure 1, the best signals were obtained with Fe (III) ion. All experimental variables were optimized in order, after good signals were obtained with Fe (III) ions.

<Figure 1>

3.1.Effect of pH and Buffer Volume

Solution acidity plays an important role in the CPE process when especially ionizable compounds are extracted to micellar medium. In the CPE, the ionic form of a neutral molecule normally does not interact and bind with the micelle aggregate as strongly as its unionized form does.[27] However, changing the pH will change the ionization form of certain analytes and other components in the medium and will

thereby affect their solubility in the water and abilities of extraction. Of course, this circumstance is related with molecular structures of analyte and surfactant. The ionic form of a neutral molecule is formed upon deprotonating of a weak acid or protonation of a weak base normally does not interact with and bind the micellar aggregate as strongly as its neutral form does.

The effect of pH on the CPE efficiency of folic acid was studied in range of pH 6.0-10.0 and the results were shown in Figure 2.

<Figure 2>

As can be seen in the Figure 2, the best signals were obtained at pH 8.0. Folic acid has four acidic ionization constant; pK_{a1}:2.29, pK_{a2}:3.50, pK_{a3}:5.05, and pK_{a4}:8.14, respectively.[28] It needs to ionize all protons of folic acid to form strong complexes with Fe (III). So, better signals were obtained beyond pH 8.0.

The experiments were repeated by using various buffer system at pH 8.0 such as borate, phosphate, and tris buffer system. According to experimental results, better signals were obtained with tris buffer than other buffer systems. So, subsequent studies were made by pH 8.0 tris buffer. After suitable pH and buffer type were selected using a series buffer solution, the concentration of buffer (0.1 M) was studied in the range 0-7 mL in the 50 mL of final volume. According to experimental results, the best signals were obtained using 2 mL buffer solution in the final volume of 50 mL.

3.2.Effect of Fe(III) Concentration

As mentioned in the previous sections, folic acid could be passed to surfactant-rich phase itself. But the signals obtained from this transfer and pre-concentration factor

were also lower than expected. The various metallic ions were tried in order to increase the signals and the best signals obtained using Fe (III) as shown in Figure 3. In order to find the optimal Fe (III) concentration on the proposed method, the effect of Fe (III) concentration was studied in the range of 0-30 mg L^{-1}.

<Figure 3>

As can be seen in the Figure 3, the obtained signals increased until 16 mg L^{-1} and decreased after this concentration. So, 16 mg L^{-1} of Fe(III) was used in the subsequent experiments.

3.3.Effect of Ionic Surfactant Concentration

In the CPE experiments, the usage of second surfactant may increase the yield of pre-concentration. Sometimes, an ionic surfactant acts as a secondary ligand and balances ionic charges in equilibrium. So, more target molecules can be passed to surfactant-rich phase. By considering this effects, various ionic surfactants were tried in order to obtain more quantitate results. Two cationic (CPC and CTAB) and one anionic surfactant (SDS) were used in the experiments. As a result of experimental studies, it was observed that preconcentration of folic acid were not affected or increased by ionic surfactants as expected. So, we decided not using ionic surfactant in order to simple the procedure.

3.4.Effect of Nonionic Surfactant Concentration

To our knowledge and literature, the high background absorbance is produced by many surfactants in the UV region can interfere with the determination of analyte by

HPLC–UV system, and this problem can be solved by using surfactants that do not have absorption peak at the wavelength of the targets, adding a mobile phase that contains methanol to the surfactant [29] and applying a back-extraction procedure to remove the surfactant. [30] According to Ren et al., Triton X-100 has strong UV absorption above 210 nm and will not suitablefor the low content of target molecules. [31] PONPE 7.5 does not absorb at 284 nm and will not interfere in determination of folic acid. So PONPE 7.5 was selected for this work

The extraction efficiency is maximized in a successful CPE procedure through minimizing the phase volume ratio. The extraction efficiency of relatively apolar organic compounds may reach 100 % even when very low surfactant concentrations to be used. The preconcentration factor is defined as the volume ratio before and after phase separation. This can be regarded as an indicator on the increases of the concentrations of analytes in the surfactant-rich phase. [32]

The effect of the PONPE 7.5 concentration on CPE of folic acid was evaluated by varying the surfactant concentration in the range of 0.00-0.15 % (w/v). At low concentration of nonionic surfactant, the extraction efficiency is decreased probably due to the inadequacy of the surfactant assemblies to entrap the hydrophobic molecules quantitatively. As it can be seen from Figure 4, the measured absorbance reached its maximum at higher concentrations above 0.12 % (w/v) of PONPE 7.5, indicating that quantitative extraction by CPE was obtained. Therefore, this concentration was selected as optimum amounts of nonionic surfactant for subsequent uses.

3.5.Effect of Equilibration Temperature and Incubation Time

Optimal equilibration temperature and incubation time are necessary to complete reactions, and achieve an easy and efficient phase separation and preconcentration. It was desirable to employ the shortest equilibration time and the lowest possible equilibration temperature as a compromise between completion of extraction and efficient separation phase. The dependence of extraction efficiency on equilibration temperature and time were studied with a range of 20–60˚C and 5–80 min, respectively. The results show that an equilibration temperature of 45 ˚C and a time of 10 min were adequate to achieve quantitative extraction.

3.6.Selection of the Diluent Agent for the Surfactant Rich Phase

In order to facilitate the sample introduction to HPLC auto sampler, it is necessary to decrease the viscosity of the surfactant-rich phase (SRP). An ideal solvent for this purpose must completely dissolve the surfactant rich phase and be also suitable to detection system. The used solvents were selected by considering their solvation power and acidic properties. After CPE, 1 mL of solvents were added into tubes in order to dilute SPR phase. A mixture of various solvents with methanol were tried at 1:1 ratio. As can be seen in Figure 5, the best signals were obtained with a mixture of 1 mol L^{-1} HCl and Methanol at 1:1 ratio. So, subsequent studies were maintained by using this solvent.

The pre-concentration factor directly is effected by volume of solvent. So, it needs to optimize for high extraction efficiency. The used volume should be minimal levels as possible in order to obtain maximum preconcentration factor. As expected, the preconcentration factor decreases with increasing solvent volume. The minimal volume requiring for HPLC micro vials is 100 µL. But it is difficult to solve and filtrate the SRP lower volumes than 200 microliter. The effect of solvent volume was investigated in the

range of 200-1500 μL. As can be seen in Figure 6, the maximum analytical signals were obtained by using 300 μL of solvent mixture.

<Figure 6>

3.7.Effect of Ionic Strength

The effect of ionic strength can be discussed in two main chapters. Firstly, most of the real samples have complex matrix components. So, the application of newly developed method in the presence of concentrated electrolytes provides that the method can be applied to real samples without any negative effect of common ions. The second approach is related with salting-out effect. It is known that the presence of electrolytes decreases the cloud point temperature and increases efficiency of separation.[33] The salt concentration is also a key parameter in CPE. The cloud point of micellar solutions can be altered by salt addition, presence of alcohol, other surfactants, polymers, and some organic or inorganic compounds, which can cause an increase or decrease on the phase micellar solubility.[34-35]

The addition of an inert salt to the samples can influence the extraction/pre-concentration process since it can alter the density of the aqueous phase. In order to study the effect of electrolyte on CPE of folic acid molecules, NaCl solution was investigated as electrolyte in the range of 0.0- 2.7 (w/v) %. The results show that addition of NaCl does not have an important effect on CPE experiments until a concentration of 2.7 (w/v) % or 0.34 mol L^{-1}. As can be seen in Figure 7, there isn't an important effect of ionic strength on developed method. These results shows that the

proposed method can be applied to samples with high ionic strength without any negative effect.

3.8.Effect of Interfering Ions

One of the biggest problem in the analysis of real sample is existing of interferences species. In the chromatographically measurements, an efficiency separation provided by analytical column and selectivity obtained from detector are limited most of interferences or decreases their effects on results. But, some species can be caused negative effect on complex formation reaction between Fe (III) ions and folic acid and yield of CPE may decrease dramatically due to these effects. When a new method was developed, possible interferences are selected according to matrix components of target samples.

Under the optimized conditions, interference studies were carried out by individually spiking gradually increased amounts of foreign interfering species into the standard solution containing folic acid at level of 100 ng mL^{-1} before CPE, and a deviation greater than ±5.0 % from the signals observed in absence of any foreign ions was used as the criterion of interference occurring. Table 1 shows the tolerance limits of the diverse species. As can be seen in Table 1, the main matrix components of food samples are not interfered the proposed method.

(Table 1)

3.9.Analytic Performance Properties of the Proposed Method and Applications

Newly developed method was optimized in detail and its analytical merits were determined by using standard solutions. The obtained data were illustrated in Table 2.

The merits were presented together before and after CPE. So, contribution or successful of the proposed method can be understood more effectively. As can be seen from the Table 2, determinations of vitamin B9 (folic acid) at trace levels can be carried out by means of high preconcentration and enhancement factor. In addition, chromatograms obtained from folic acid analysis after CPE were presented in Figure 8. It can be understood from the figure that peak area of folic acid in surfactant rich phase is increased as proportional with concentration and spectrum of this peak is provided that this peak belongs to folic acid.

<Table 2>

<Figure 8>

Analysis of certified reference material (CRM) and recovery test were carried out for validation of new method. Three different certified reference materials (NIST-3280, ERM BD6000) were used for validation of the proposed method. The results were evaluated by student t and F test. CRM samples were prepared the procedure as explained in section 2.5. According to this method, 10 mL of the obtained solution were presented CPE experiments and their vitamin contents were analyzed by using developed method. Results for validation analysis were presented in Table 3.The average values obtained using calibration curve method are in good agreement with the certified values.

The developed method was applied to various food and pharmaceutical samples in order to determine their contents of folic samples. For this purpose, samples were prepared as explained in section 2.5. In addition, 150 and 300 ng mL^{-1} of folic acid

was spiked to all samples in order to check the accuracy of method. The results of this study were given in Table 4. The results indicate that recoveries are quantitatively appropriate at reasonable levels for trace folic acid analysis in the range of 95.1-105.1 % in food samples.

(Table 4)

4. Conclusions

CPE is one of the simplest and useful preconcentration methods for trace analysis in literature. It is extensively used in trace analysis of inorganic and organic species owing to positive properties such as simplicity, green chemistry friendly, and cheaply. The proposed method renders possible this analysis using a preconcentration step prior to HPLC measurements.

The developed method is very sensitive and also selective due to complex formation between Fe(III) ions and folic acid. As far as we know, there is not a published study for folic acid based on chromatographically determination following cloud point extraction. Only, a study was published by Heydari et al. for all water soluble vitamins based on ion-pair CPE. But, the detection limits of this method was so high (μg mL^{-1} levels) according to the presented method. [36] The recommended procedure could be successfully applied to preconcentration and determination of folic acid (Vitamin B9) in a wide range of food samples.

Acknowledge

This study has been supported by Cumhuriyet University Scientific Research Projects Commission as the research project with the ECZ-004 code. Authors gratefully

thanks Prof. Dr. Şahin YILDIRIM for his useful comments and contributions to the preparation of the project text.

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Table 1. Effect of possible matrix species on CPE efficiency of folic acid (N: 5, 100 ng mL^{-1} Vitamin B9)

Interfering Species	**Tolerance Limits**
K^+, Na^+, and NH_4^+	1000
Cl^-, SO_4^{2-}, Ba^{2+}, Ca^{2+}, Acetate	750
NO_3^-, Vitamin C, PO_4^{3-}, and CO_3^{2-}	500
Cu^{2+}, Mg^{2+}, Zn^{2+}	350
Vitamin B5, Al^{3+}	300
Vitamin B3 and B12, Fe^{2+}	200
Vitamin B2	100

Table 2. Analytical characteristics of the proposed method

Parameter	Before CPE	After CPE
Linear Range	1000-50000 ng mL^{-1}	20-1200 ng mL^{-1}
Limit of Detection[a]	360 ng mL^{-1}	6.06 ng mL^{-1}
Limit of Quantification[b]	1030 ng mL^{-1}	20.18 ng mL^{-1}
RSD (%)	3.52 (25000 ng mL^{-1})	2.65 (300 ng mL^{-1})
Calibration Sensitivity	35.713	1955.7
Correlation coefficient (r^2)	0.9998	0.9976
Pre-concentration Factor[c]	-	166.7
Enhancement Factor[d]	-	56.0

[a] Based on statistical $3S_{blank}$/m-criterion for ten replicate blank absorbance measurements

[b] Based on statistical 10 S_{blank}/m-criterion for ten replicate blank absorbance measurements

[c] Preconcentration factor is defined as the ratio of the initial solution volume (50 mL) to the volume of surfactant rich phase (0.3 mL)

[d] Enhancement Factor is defined as ratio of slope of calibration before and after CPE

Table 3. The levels of Folic Acid (Vitamin B9) in the certified reference materials (CRMs) after application of the developed procedure (N: 5).

CRM	Certified Value, mg kg^{-1}	Found[a] mg kg^{-1}	% RSS	Recovery %	t_{exp} value[b]	F_{exp} value[c]
NIST 3280	394±22	386±24	6.21	97.9	0.81	1.19
ERM-BD6000	0.74±0.04	0.76±0.05	6.57	102.1	0.09	1.56

[a] The average value of five replicates ± standard deviation. [b] The tabulated t-value at 95% confidence level is 2.45 for five replicate measurements. [c] The tabulated F value at 95% confidence level is 4.28 (N=6)

NIST 3280 : Multi Vitamin Tablet

ERM-BD 6000 : Milk powder

Table 4. The amounts of folic acid (Vitamin B9) in various samples after the developed CPE procedure (N:5).

Sample	Added ng mL^{-1}	Found[a,b] µg Kg^{-1}	RSS %	Recovery %
Baby Food 1	-	445.34±15.45	3.46	-
	150.00	587.54±16.50	2.80	98.7
	300.00	738.10±17.12	2.32	99.0
Baby Food 2	-	332.21±12.64	3.80	-
	150.00	491.11±15.50	3.16	101.9
	300.00	640.40±18.11	2.83	101.3
Vitamin Tablet	-	860.48±21.55	2.50	-
	150.00	1062.34±22.32	2.10	105.1
	300.00	1185.86±26.47	2.23	103.1
Parsley	-	85.77±4.14	4.86	-
	150.00	224.14±8.85	3.94	95.1
	300.00	379.28±10.36	2.73	98.3
Wheat	-	765.30±4.02	5.25	-
	150.00	904.04±16.45	1.82	98.7
	300.00	1051.34±18.22	1.73	98.6

[a] The average value of five replicates ± standard deviation

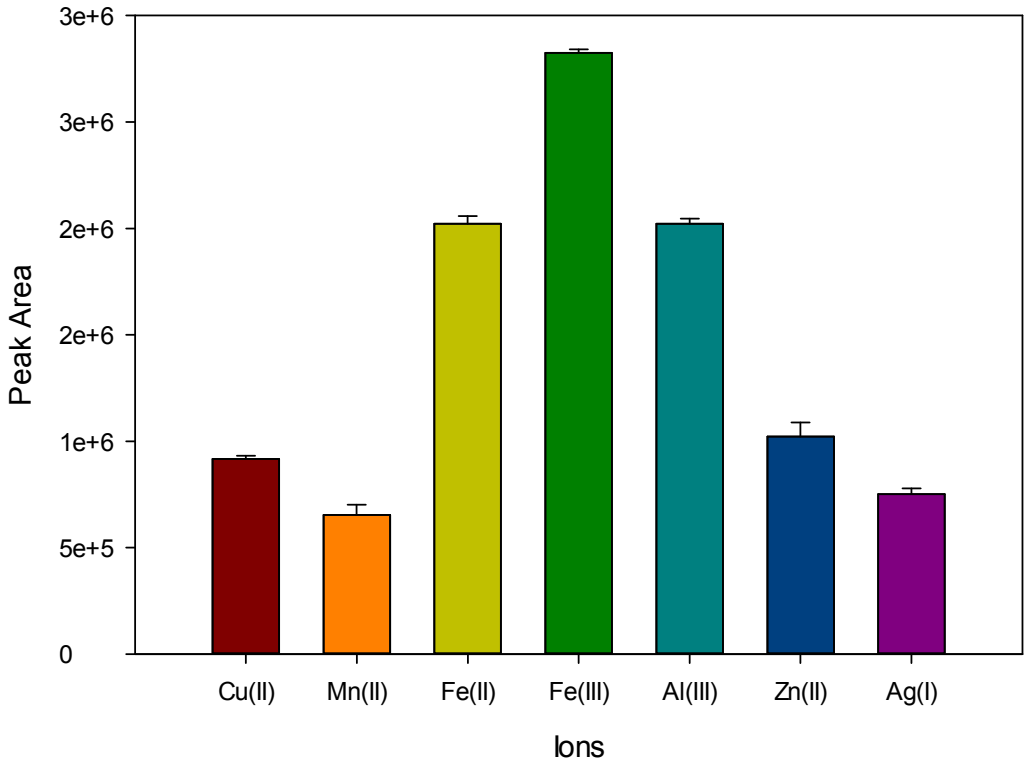

Figure.1 The effects of various ions on CPE of folic acid (n=3)

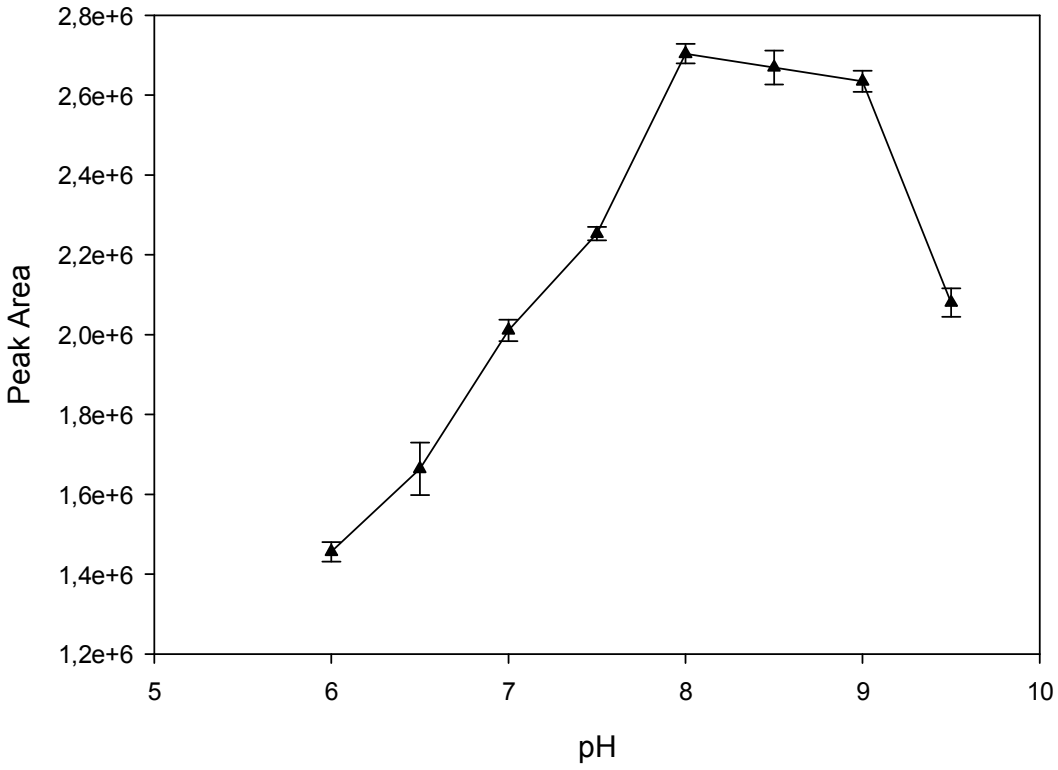

Figure 2. The effect of pH on the proposed method (n=3)

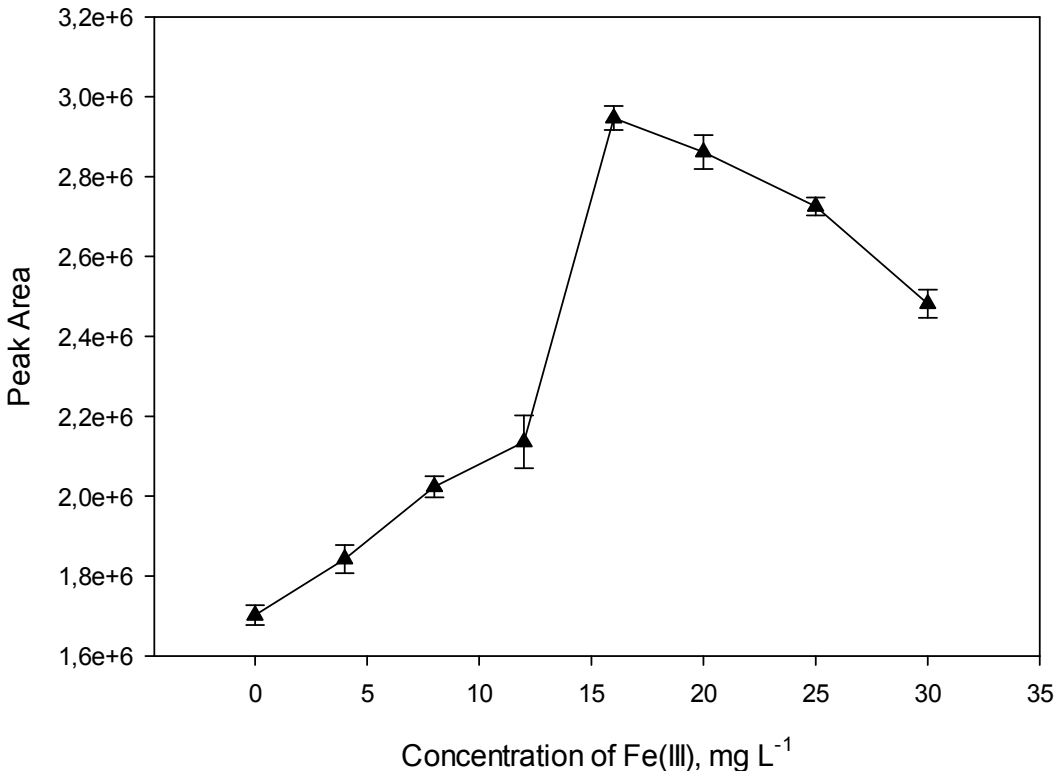

Figure 3. The effect of Fe (III) concentration on the proposed method (n=3)

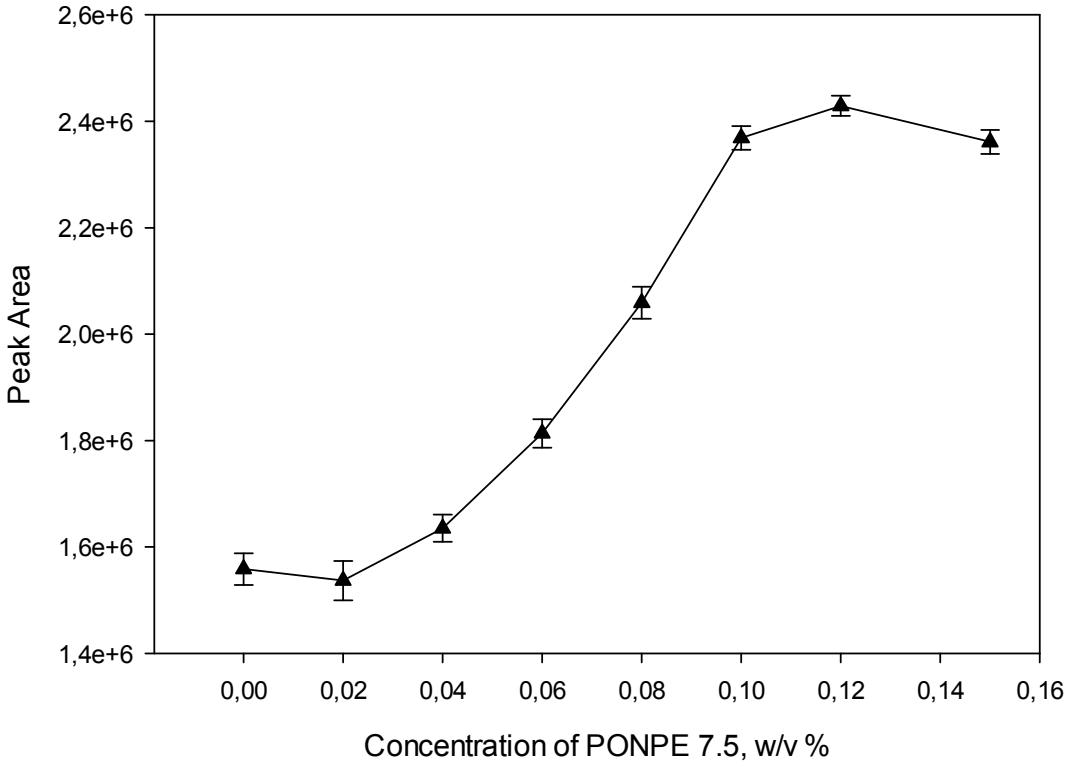

Figure 4. The effect of surfactant concentration on the proposed method (n=3)

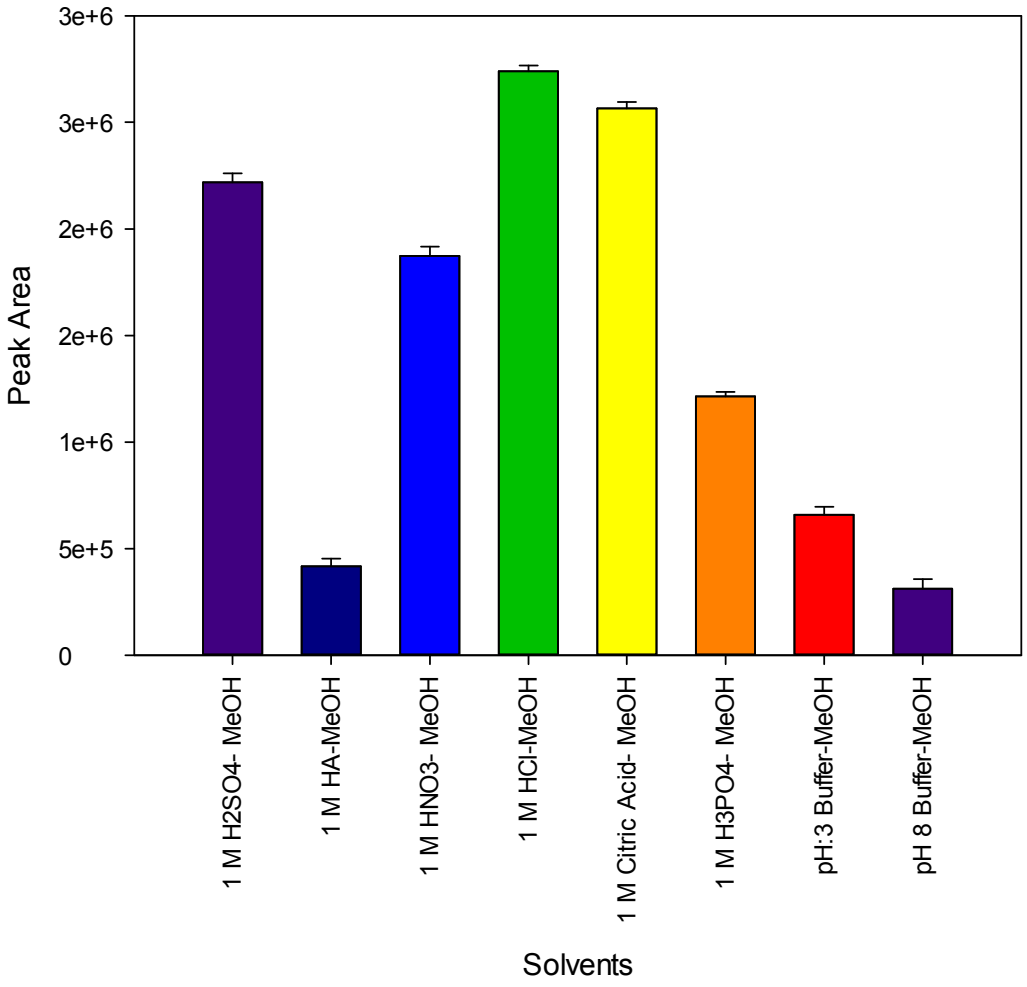

Figure 5. Effect of solvent type to dilute the surfactant rich phase (n=3)

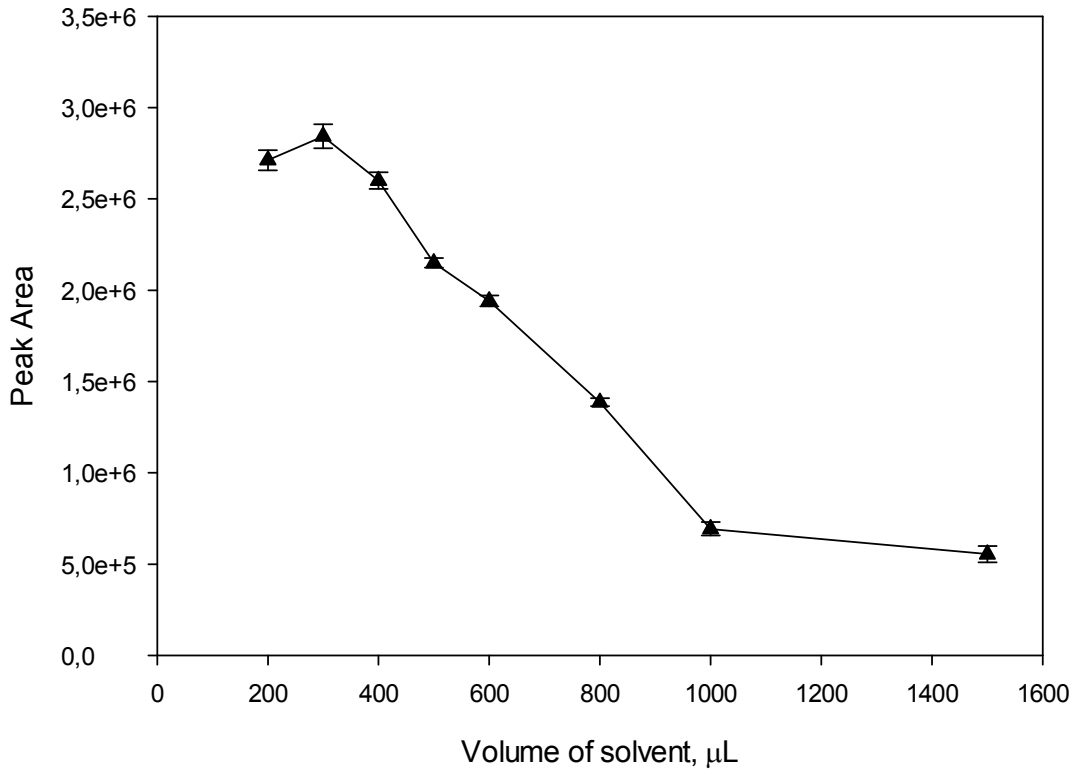

Figure 6. Effect of solvent amount for surfactant rich phase on analytical signal (n=3)

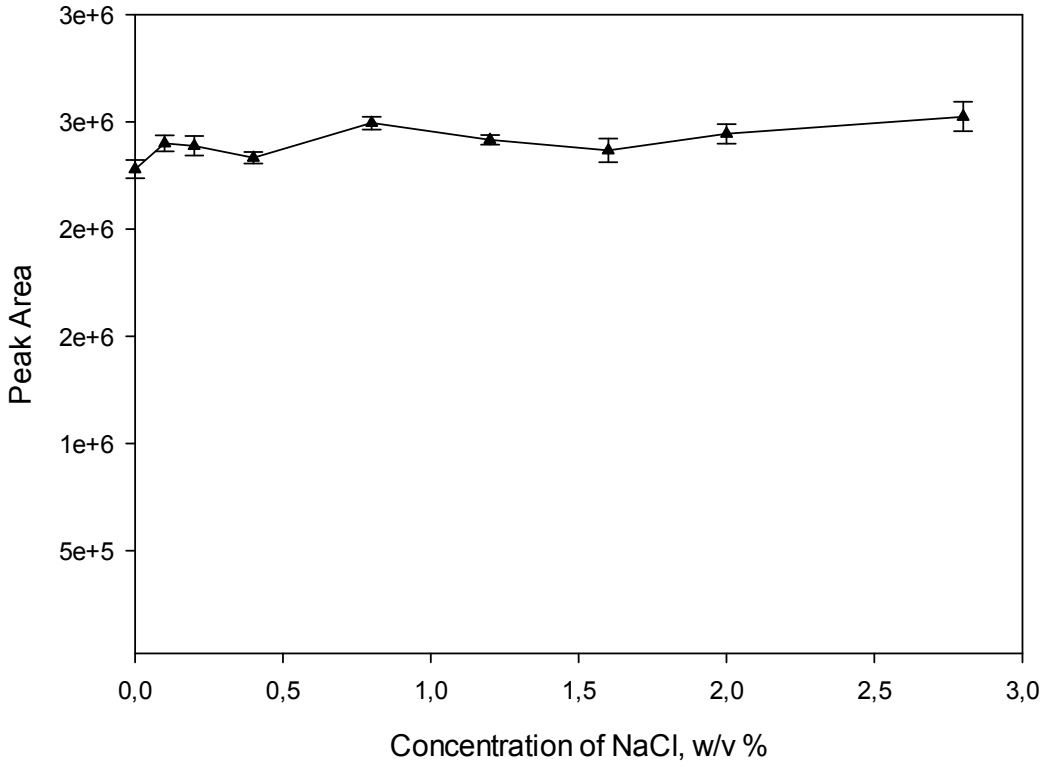

Figure 7. The effect of electrolyte concentration on the proposed method (n=3)

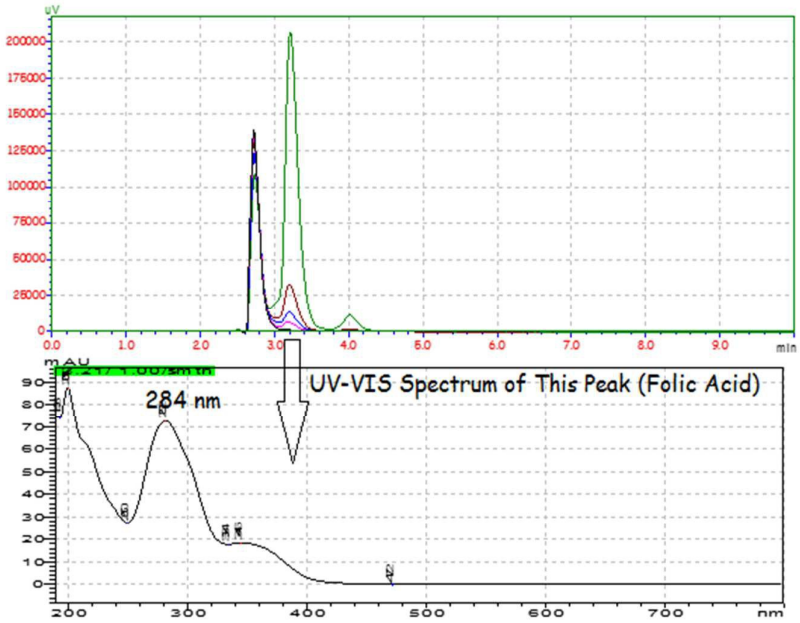

Figure 8. Chromatograms of folic acid (25.0, 50.0, 200.0, and 1000.0 ng mL^{-1}) after CPE

Parameter	Before CPE	After CPE
Linear Range	1000-50000 ng mL^{-1}	20-1200 ng mL^{-1}
Limit of Detection[a]	360 ng mL^{-1}	6.06 ng mL^{-1}
Limit of Quantification[b]	1030 ng mL^{-1}	20.18 ng mL^{-1}
RSD (%)	3.52 (25000 ng mL^{-1})	2.65 (300 ng mL^{-1})
Calibration Sensitivity	35.713	1955.7
Correlation coefficient (r^2)	0.9998	0.9976
Pre-concentration Factor[c]	-	166.7
Enhancement Factor[d]	-	56.0