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SIMULTANEOUS DETERMINATION TRACE LEVELS OF VITAMIN B_1 AND VITAMIN B_9 IN HUMAN SAMPLES BY ULTRASOUND-ASSISTED DISPERSIVE LIQUID-LIQUID MICROEXTRACTION COUPLED WITH HPLC-UV

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Keywords:

Thiamine; Folic acid; UA-DLLME; Hybrid Box–Behnken design -genetic algorithm; HPLC.

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ABSTRACT: Ultrasound-assisted dispersive liquid-liquid micro extraction (UA-DLLME) was proposed for simultaneous determination of trace levels of Vitamin B_1 (thiamine) and Vitamin B_9 (folic acid) in the human urine and serum samples. High performance liquid chromatography (HPLC) coupled with chemometrics method was employed to analysis the extraxtants. **Objective:** The optimal conditions of the extraction recovery, including pH of sample solution, type and volume of extraction solvent, disperser solvent type and volume, temperature and time of ultrasound, centrifugation time and ionic strength were were studied step-by-step via one-factor-at-a time procedure. Then significant variables were chosen and considered to further optimize using the hybrid Box–Behnken design -genetic algorithm. UA-DLLME method with HPLC-UV and utilizing hybrid Box–Behnken design -genetic algorithm to optimize the extraction process was presented for the first time in this study. Under the optimized extractions, relative standard deviations (RSD) of the analyses less than 4% (n= 3) and detection limit of 3.09-11.15 ng mL. Recoveries for both thiamine and folic acid were in the ranges of 88–107%. As the result, the UA-DLLME – HPLC coupled hybrid Box–Behnken design -genetic algorithm was successfully applied for the simultaneous determination trace levels of thiamine and folic acid in human urine and serum samples.

INTRODUCTION: B Vitamins are water-soluble Vitamins, which are necessary to a healthy diet as they are involved in tissues growth, normal cells function [1]. Thiamine is also known as Vitamine B_1 [2] and folate is a general type of water soluble B Vitamins, also known as B_9 [3]. Thiamine and folic acid are types of B Vitamins that are very necessary for body functions, conduction of brain and nerve signals [4].

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Determination of thiamine and folic acid are great of clinical importance in various patients. Several chromatographic methods have been reported for analyzing of folates in human urine and serum. Several methods have been reported for determination of B Vitamins such as including radioimmunoassay [5, 6], capillary electrophoresis [3, 7-8], liquid chromatography–mass spectrometry (LC–MS), and liquid chromatography–tandem mass spectrometry (LC–MS/MS) [9–14].

Among these methods HPLC is considered as a sensitive and selective method and therefore suitable for active substance determination; it is also considered as a great tool for evaluating the formulation stability in cosmetic and pharmaceutical industries [13-14].

DLLME method was reported by Assadi and developed by Rezaee et al., (2006) [15] which is an effective microextraction technique for preconcentration and separation of organic and inorganic specimens. This method is a ternary component solvent system that applies a mixture of a water-immiscible extraction solvent dissolved in a water-miscible disperser solvent injected rapidly into an aqueous sample. DLLME has a higher enrichment factor, is safe for use with hazardous samples, cost effective, environmental friendly and easier to incorporate into automated analytical methods [16-18]. After extraction, sample must be centrifuged to facilitate phase separation. Ultrasonic radiation accelerates mass transfer processes in the ultrasound-assisted dispersive liquid–liquid microextraction (UA-DLLME).

George E. P. Box in the 1950s introduced response surface methodology (RSM) -a factorial design based method for collection of statistical techniques- which has been used for modeling and optimization of some chemical and physical processes [19]. Different classes of RSM such as central composite design (CCD), Box-Behnken design (BBD) and three-level factorial design have different properties and characteristics. Response surface methodology (RSM) is a collection of mathematical and statistical techniques for empirical model building. Response surfaces are used to determine optimal experimental conditions [20]. In addition, it is a good way to graphically illustrate the relation between different experimental variables and the response(s).

Genetic Algorithms (GA) are powerful stochastic search algorithms to find solutions for optimization problems based upon the Darwinian evolution hypothesis [21-22]. GA are powerful stochastic search algorithms to find solutions for optimization problems based upon the Darwinian evolution hypothesis [22].

The optimal conditions of the extraction recovery, including pH of sample solution, type and volume of extraction solvent, disperser solvent type and volume, temperature and time of ultrasound, centrifugation time and ionic strength were optimized. Then significant variables were considered and optimized using the hybrid Box–Behnken design -genetic algorithm.

This method aimed to investigate and validate a method for the simultaneous determination of thiamine and folic acid concentrations in human urine and serum samples by HPLC after optimization by UA-DLLME coupled with chemometrics method was employed to analysis the extraxtants.

1. Instrumentation and software: HPLC (Shimadzu, LC20A System), vacuum degasser and system controller (SCL-10Avp) were used for testing. Chromatographic separation was conducted on a C18 (250mm×4.6mm, 5µm) column. UV detection was performed at 200 to 300 nm with a spectral resolution of 1.0 nm and integration period of 0.4 s per spectrum. The mobile phase was consisted of methanol, Water and 0.1% trifluoroacetic acid (TFA) (pH=4.0) buffer solution was delivered at 1mL/min. The pH was measured using a pH meter (Metrohm 827, Switzerland) combined with a glass electrode. Before use, the pH meter was calibrated against standard Merck buffers (pH = 4.0 and pH = 7.0). The sample (20 µL) was injected into the HPLC with a syringe (Hamilton). A 320R Hettich centrifuge (Germany) and a digital 10P ultrasonic bath (Sonorex; Germany) was used.

2.2. Chemicals and reagents: The analytical-reagent grade of Vitamins (>99%) was purchased from Sigma Aldrich (Steinheim, Germany). The stock solutions of Vitamins were prepared by dissolving in water. The working solutions were prepared by appropriate dilution of the stock solution with double distilled water. Double distilled deionized water was produced by a Milli-Qsystem (Millipore, Bedford, MA, USA). Standard solutions stored at 4 °C and brought to ambient temperature just prior to use. In throughout the experimental runs all the solvents, calibration and real samples were filtered through 0.45 µm nylon filter membranes (Varian, USA).

2. Extraction method: Human urine and serum samples were prepared using the UA-DLLME method. Aliquots of 1 mL human urine sample were alkalinized with 200 µL (NaOH 1 mol L^{-1}) for the hydrolysis of acyl glucuronic acid conjugates and then neutralized with 200 µL (HCl 1 mol L^{-1}).

To an aliquot of 100 µL of human serum sample, a solution containing 50 µL of 10% TCA was added for protein precipitation. This mixture was shaken for 10 s and then filtrated through 0.45 µm nylon filter membrane. The filtrate was analysis with the procedure described below.

Standard stock solutions were prepared by dissolving Vitamin in deionized water and working standard solutions at different concentrations were prepared freshly by mixing the appropriate volumes of the stock solutions and diluting with deionized water. The real samples in this study were collected from human urine samples and then stored at 4-5°C until analysis. Human urine samples were prepared using the UA-DLLME method. Aliquots of 0.5 mL human urine sample were alkalinized with 200 µL (NaOH 1 mol L^{-1}) for the hydrolysis of acyl glucuronic acid conjugates and then neutralized with 200µL (HCl 1 mol L^{-1}). The samples were placed in centrifuge glass vials and their ionic strength and pH were adjusted to the optimum level (pH 3.0).100µL tetrachloride (as extracting solvent) and 50 µL methanol (as dispersive solvent) into a sample solution. The vial was immersed in an ultrasonic water bath, sonicated for 3 min, and shaken manually. A cloudy solution formed in the test tube and then the mixture was gently shaken and the cloudy solution was centrifuged for 4 min at 5500 rpm. After slowly discarding the aqueous solution, the resulting droplet and lipidic solid were dissolved at 20 µL with HPLC grade methanol and then filtrated through a 0.45 µm membrane to throw out the white floccule from the extract of samples.

RESULTS AND DISCUSSION:

1. Chromatographic conditions: The variables affected by the chromatographic conditions such as pH, and solvent ratios was studied and optimized. In this study, the effect of pH on the extraction was investigated over the pH range of 2.0-5.0. The pH level was found to be important to the separation process. It was found that the higher pH (5.0) and lower pH (2.0) values would increase the tailing of the peak and decrease the resolution. A pH of 2.5 was chosen as the optimum value for better resolution, better peak shape and a short run time. Various ratios of solvents were tested. It was found that in the mobile phase the ratio of methanol and water affected the symmetry of the peak shapes. Thus mobile phase containing a water/methanol mixture phosphate buffer solution at pH = 2.5, flow rate of 1.0 ml min^{-1} were used in this study. An elution gradient was chosen that allowed complete analysis in less than 5 min.

2. Optimization variables using one-at-a time method: In order to optimize the experimental variables on the extraction recovery, two methods were applied. In the first stage, traditional optimization method varying one variable at-a-time was used for screening the several factors to determine significant factors. After screening out the factors with insignificant effect, the remaining factors were optimized using the Box–Behnken design.

3. Effect pH of sample solution: The pH of sample solution is an important factor and can be affected on the extraction recoveries [15]. There for the effect of pH on the recoveries of Vitamins was investigated in the range of 3.0-5.0. According to the obtained results, the extraction recovery of Vitamins was increased when the sample pH was decreased to 3.5. This is due to the fact that these Vitamins were not in ionic form at low pH (revert of dissociation). Finally, pH of 3.5 was chosen as the optimum pH sample solution for the following experiments. The results are shown in **Fig. 1.**

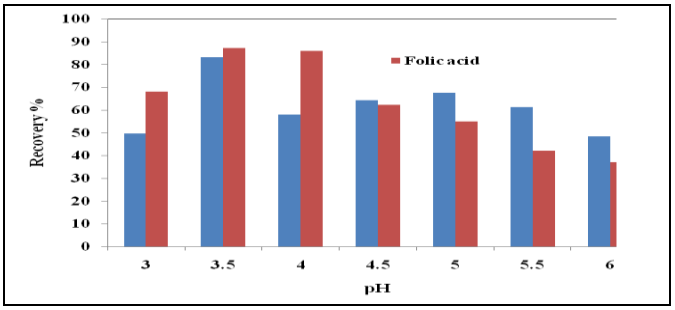


FIG. 1: EFFECT OF pH SAMPLE SOLUTION ON THE RECOVERIES OF EACH VITAMIN.

4. Effect of type and volume of disperser solvent and extraction solvent: Different solvents including acetonitrile, methanol, ethanol and acetone (as dispersive solvent) and carbon tetrachloride, chloroform, chlorobenzene, and dichloromethane (as extractant solvent) were tested. The disperser solvent would influence the formation of the cloudy solution and the produced infinite interface increases the mass transfer. Among tested solvent as disperser methanol showed the highest recovery and more stable

cloudy solution. The suitable extraction is very important for the DLLME method. The extracting solvent has to meet some properties such as low and high solubility in water and high extraction capability of the target analyte. Therefore, carbon tetrachloride was selected as extracting solvents because it had higher recoveries in comparison with the other solvents.

In order to obtain the highest extraction efficiency of the DLLME method, the volume of the solvent had to be optimized. Optimization process was performed using various volumes of methanol and carbon tetrachloride in the range of 10.0 to 60.0 µL and 50.0 to 120.0 µL respectively. Finally, the volume of methanol 50.0 µL and carbon tetrachloride 100.0 µL was chosen as the optimal volume for further investigations. The results are shown in **Fig. 2 a-b.**

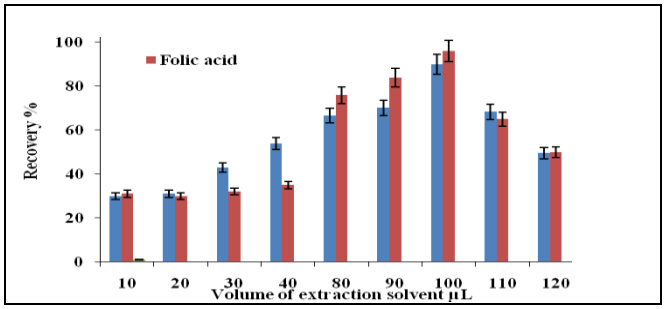


FIG. 2.a

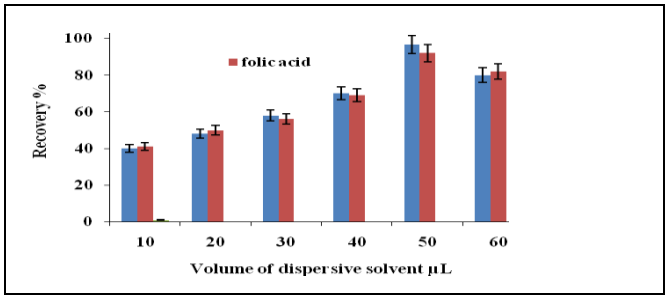


FIG. 2.b

FIG 2: EFFECT OF EXTRACTION SOLVENT VOLUME (a) AND DISPERSIVE SOLVENT VOLUME (b) ON THE RECOVERIES OF VITAMINS. CONDITIONS: SAMPLE SOLUTION, 5 mL (50 ng mL^{-1}) OF EACH VITAMINS; pH SAMPLE SOLUTION: 3.5.

5. Comparison of ultrasound-assisted and shake-assisted liquid–liquid microextraction: In this method, for improve the homogeneity, ultrasound- and shaking-assisted methods were investigated with a series of experiments. The results shown in **Fig. 3.a** reveal that recovery (i.e., extraction efficiency) of analytes with ultrasound-assist are higher than with shake-assist. Comparison with shake-assisted extraction. The result also indicate that the formation of emulsion increases extraction efficiency for analytes when using ultrasound-assisted approach. To accelerate the mass transfer processes, increase extraction efficiency and reduce extraction time, ultrasonic radiation is used in ultrasound-assisted dispersive liquid–liquid micro extraction (UA-DLLME). Effect of ultrasonic time on the extraction recovery was examined in the range of 0-5 min. The maximum recovery was obtained for ultrasonication of 4.0 min and no improvement was achieve by further ultrasonication. It was probably due to the fact that the ultrasonic water bath could generate the emulsion quickly and rapidly make a very large contact surface area between the extraction solvent and the aqueous phase. Therefore, 4 min was found to be the optimum extraction time. The results are shown in **Fig. 3.b.**

Temperature affects solubility of organic solvent in water as well as the distribution coefficients and mass transfer of target analytes. The effect of extraction temperature on the extraction recovery was evaluated over the range of 15-35 ºC. The results are shown in **Fig. 3.c.** Maximum extraction recoveries were obtained at 25ºC for the both analytes. As can be seen in **Fig. 3b**, extraction recoveries decreased at lower and higher temperatures. Finally, 25ºC was taken to be the optimum extraction temperature.

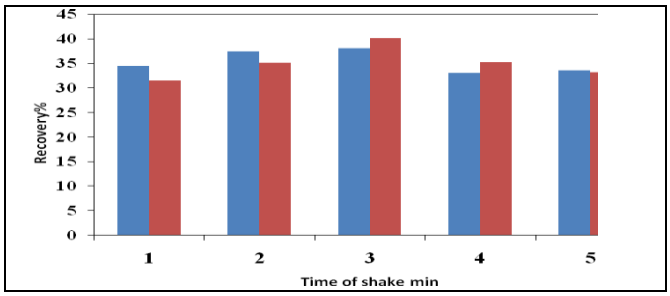


FIG.3.a

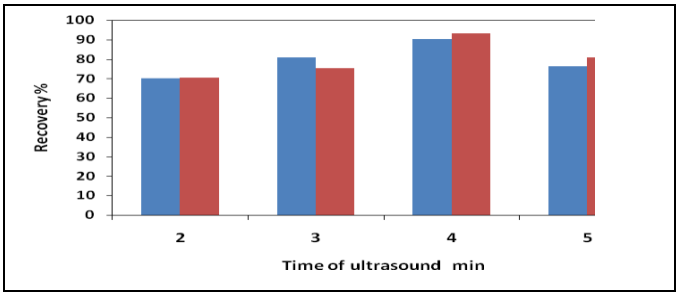


FIG.3.b

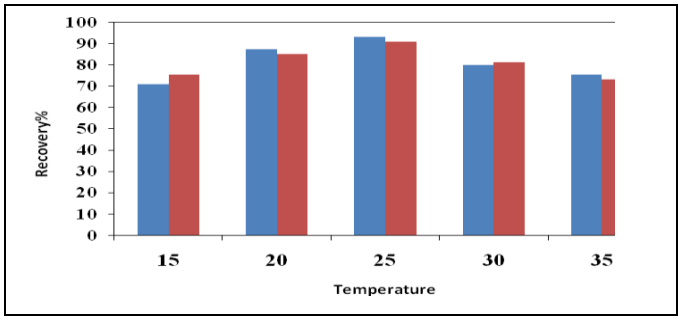


FIG.3.c

FIG. 3: COMPARISON OF ULTRASOUND-ASSISTED DLLME AND SHAKE-ASSISTED-DLLME FOR EXTRACTION EFFICIENCY (a), EFFECT OF TIME (b) AND TEMPERATURE (c) OF ULTRANSOUND ON THE RECOVERIES OF VITAMINS. CONDITIONS: SAMPLE SOLUTION, 5 mL OF (50ng mL^{-1}) OF EACH VITAMINS; pH SAMPLE SOLUTION: 3.5; VOLUME AND TYPE OF EXTRACTANT SOLVENT: CARBON TETRACHLORIDE, 100.0 µL AND VOLUME AND TYPE OF DISPERSIVE SOLVENT: METHANOL, 50.0µL, TIME AND TEMPERATURE OF ULTRASOUND: 4 MIN, 25 ºC.

6. Effect of centrifugation time and rate: Centrifugation was required to break down the emulsion and accelerate the phase-separation process. In this method, extraction time is defined as interval time between injection of the mixture solvents (the dispersant and the extractant) to the sample and starting to centrifuge. The effect of extraction time was examined in the range of 0-5 min 3000-6000 rpm. Therefore 4 min 5500 rpm were selected as the optimum centrifugation time and rate. The results are shown in **Fig. 4.**

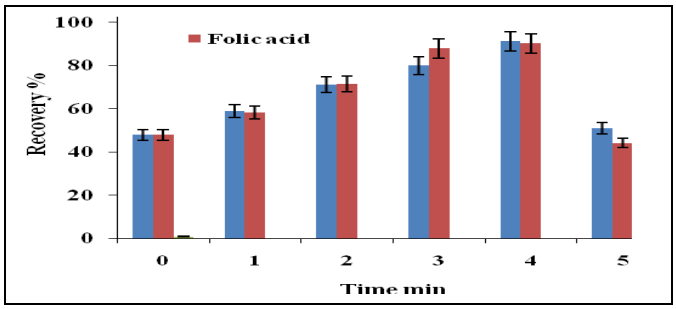


FIG. 4: EFFECT OF CENTRIFUGATION EXTRACTION TIME ON THE RECOVERIES OF VITAMIN. CONDITIONS: SAMPLE SOLUTION, 5 mL OF (50ng mL^{-1}) OF EACH VITAMINS; pH SAMPLE SOLUTION: 3.5; VOLUME AND TYPE OF EXTRACTANT SOLVENT: CARBON TETRACHLORIDE, 100.0 µL AND VOLUME AND TYPE OF DISPERSIVE SOLVENT: METHANOL, 50.0µL; ULTRASOUND EXTRACTION TIME: 4 MIN.

7. Ionic strength: The addition of sodium chloride to samples has been used to improve the extraction efficiency. The influence of ionic strength on the performance of DLLME method was investigated by adding different amounts of NaCl (0.0, 0.05, 0.10, 0.15 and0.20 g). The results indicated that the extraction efficiency decreases, with addition of salt up to 0.05 g salt content. Therefore, NaCl 0.05 g was used in the subsequent experiments. The results are shown in **Fig. 5.**

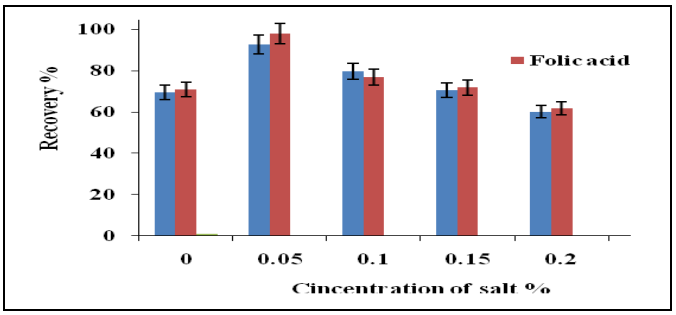


FIG. 5: EFFECT OF IONIC STRENGTH ON THE RECOVERIES OF VITAMINS. CONDITIONS: SAMPLE SOLUTION, 5mL OF (50 ng mL^{-1}) of EACH VITAMINS; pH SAMPLE SOLUTION: 3.5; VOLUME AND TYPE OF EXTRACTANT SOLVENT: CARBON TETRACHLORIDE, 100.0 µL AND VOLUME AND TYPE OF DISPERSIVE SOLVENT: METHANOL, 50.0 µL; ULTRASOUND EXTRACTION TIME: 4 MIN; CENTRIFUGATION TIME AND RATE: 5500 RPM AND 4 MIN.

8. Optimization of the variables using hybrid Box–Behnken design -genetic algorithm: According to the one-at-a time method, four independent variables, including pH (A), dispersion volume (B), extraction volume (C), and time of ultransound (D) were studied further by Box-Beh design. For each parameter, three levels were assigned and coded by (-1, 0, +1). To make the model and estimate the optimum of variables we used Minitab software that designed a set of experiments according to Box–Behnken design (BBD). This design led to study the effects of the four variables in a three block of 27 sets of test conditions and three central points were selected for each set of experiments to estimate the error. Independent variables and their levels for the hybrid Box–Behnken design used in this study are shown in **Table 1** and **Table 2.**

TABLE 1: FACTORS AND THEIR LEVELS IN BOX BENKEN DESIGN.

Factors	Level		
	Low	Center	High
	-1	0	1
pH A	3	3.5	
$V_{disperser}$ µL B	40	50	60
$V_{extraction}$ µL C	90	100	110
Time* D	3	4	5

* Time of ultransound

TABLE 2: FACTORS AND THEIR LEVELS IN BOX–BEHNKEN EXPERIMENTAL DESIGN AND OBTAINED RESULT FOR EACH RUN.

Run no.	Coded variables levels				Absorbance
	A	B	C	D	
1	-1	-1	0	0	0.860
2	1	0	0	1	0.652
3	0	0	1	1	1.098
4	0	0	1	-1	0.999
5	1	1	0	0	0.642
6	-1	1	0	0	0.881
7	0	0	-1	-1	1.003
8	0	-1	0	1	0.996
9	0	1	-1	0	1.001
10	-1	0	-1	0	0.885
11	0	0	-1	1	1.007
12	0	-1	1	0	0.989
13	0	-1	0	-1	0.994
14	0	1	0	-1	1.003
15	0	1	0	1	1.012
16	1	-1	0	0	0.664
17	1	0	-1	0	0.659
18	0	0	0	0	1.022
19	0	0	0	0	1.007
20	0	-1	-1	0	0.994
21	1	0	0	-1	0.555
22	-1	0	1	0	0.863
23	0	0	0	0	1.036
24	1	0	1	0	0.645
25	0	1	1	0	1.031
26	-1	0	0	1	0.872
27	0	-1	1	0	0.544

The response surface model was developed by considering all the significant interactions in the BBD. The response variable (Y) and the tested variables were related by the following second-order polynomial equation (Eq. (1)).

$$Y=1.021-0.1187\ A-0.0060\ B+0.0063C+0.0168\ D-0.2611\ A^2-0.010\ B_2^2$$

$$+0.0034\ C^2-0.0095\ D^2-0.0105\ A*B+0.0020\ A*C+0.0262\ A*D$$

$$+0.0087\ B*C+0.0015\ B*D+0.0237\ C*D \quad (1)$$

$n=27$, $R^2=0.9878$, PRESS=0.042.

Analysis of variance (ANOVA) was applied to evaluate the statistical significance of the model. If p-value (smallest level of significant) was lower than 0.05, it indicates that the model would be statistically significant. The summery of the ANOVA is shown in **Table 3.** The fitness of the model equation was evaluated by the regression coefficients (R^2) of determination thiamine and folic acid. The value of R^2 (0.9878) also indicated a good agreement between the experimental and predicted values of response. F-test was used to estimate the statistical significance of all terms in the polynomial equation within 95% confidence interval [19-20]. The lack of fit (LOF) is a special test assesses goodness of fitting the data from the model. The Lack of fit report only appears when it is possible to conduct this test. A p-value of LOF i.e. 0.230 indicates the good ability of model and best selection of optimum conditions.

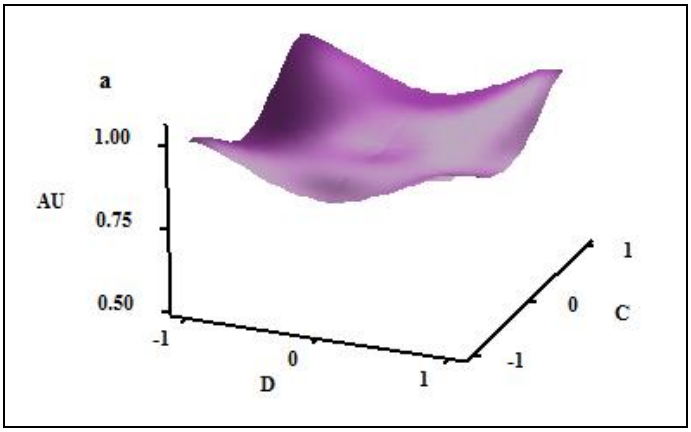
TABLE 3: ANALYSIS OF VARIANCE (ANOVA) FOR BOX–BEHNKEN DESIGN.

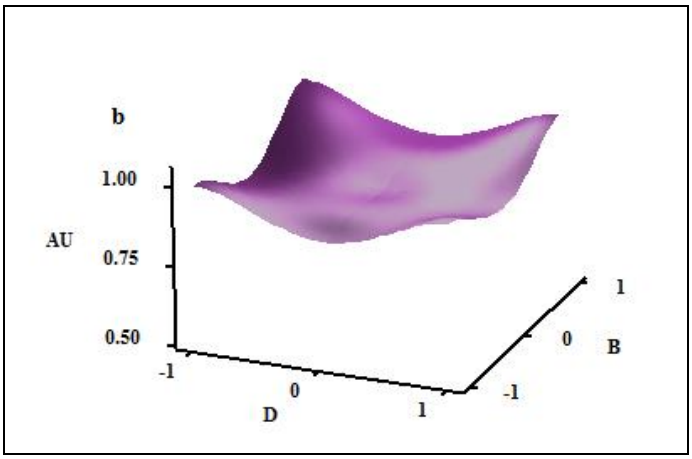
Source	DF	SS	MS	P-Value
Regression	14	0.6240	0.0455	0.0
Residual	12	0.0076	0.0006	
Lack-of-Fit	10	0.0073	0.0007	0.230
Pure Error	2	0.0003	0.0001	

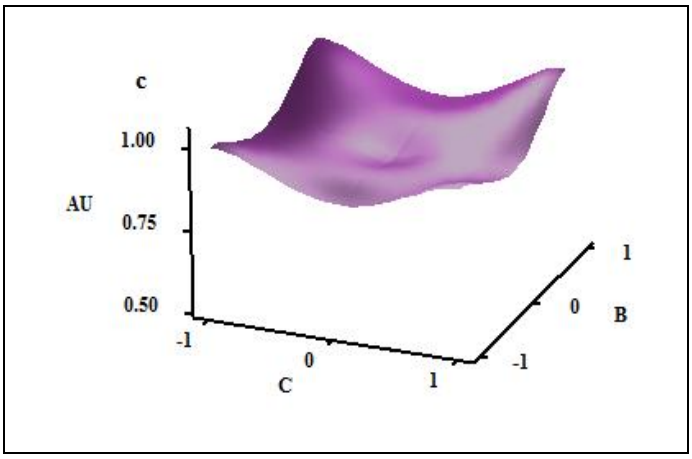
SS =sum of squares; DF =degrees of freedom; MSS =mean sum of squares; p-Value = probability value.

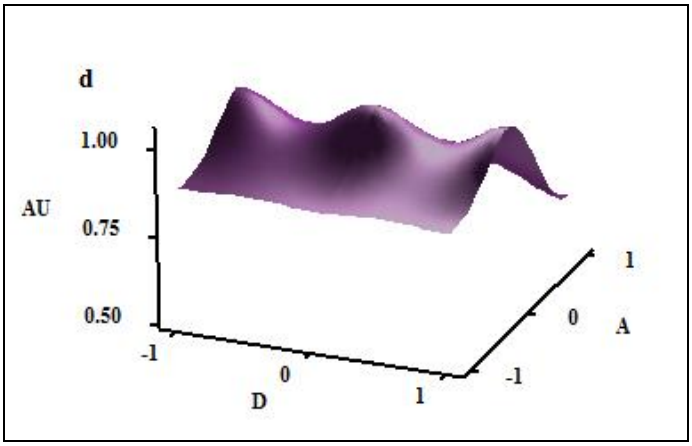
The main and interaction effects of variables can be estimated from the outputs of experimental design which is the main reason of doing experimental design. The extraction solvent had significant effect on the absorbance. The appearance of quadratic terms of extraction solvent in the eq. 1 indicates that the absorbance increases sharply as volume solvent decreases.

The pH of solution had significant effect on the absorbance. The appearance of quadratic terms of pH of solution in the eq. 1 indicates that the absorbance increases slowly as pH solution decreases. Response surface curves facilitate investigating the interaction between the independent variables and finding the optimal level for each variable, as well. These curves are represented in **Fig. 6 (a)–(e).** Inspecting these figures show that amounts of interactions between variables are little as the extension of curvature of the plots in not very high. In spite of this observation, the interaction terms appeared significantly in the model equation. After generation of the polynomial equations that relate the absorbance to the independent variables, the GA was used for optimization of the process. The optimum values of the tested variables found to be as follows: A= 3.4, B= 48µL, C=101 µL and D= 4.0 min.









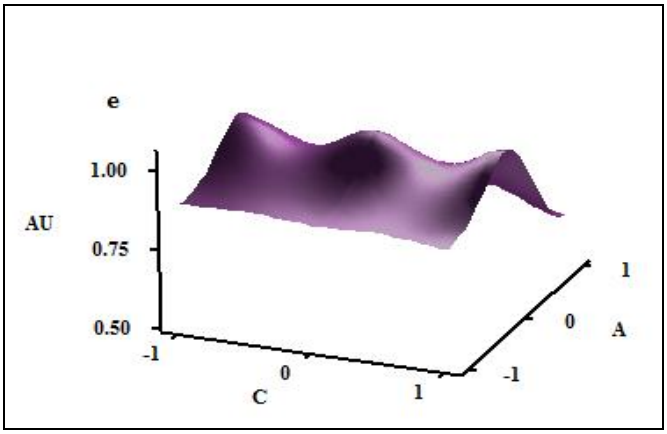


FIG. 6: RESPONSE SURFACES PLOT FOR THE BOX–BEHNKEN DESIGN (a) TIME OF ULTRANSOUND (D) - EXTRACTION VOLUME (C); (b) TIME OF ULTRANSOUND (D) - DISPERSION VOLUME (b); (c) EXTRACTION VOLUME (C) - DISPERSION VOLUME (B); (d) TIME OF ULTRANSOUND (D) - pH (A); (e) EXTRACTION VOLUME (C) - pH (A).

9. Analytical features of proposed method: Under the optimal conditions obtained above, the analytical performance of the proposed method was investigated. Analytical features including, dynamic range, sensitivity (SEN), selectivity (SEL), limit of detection (LOD), and relative standard deviations (RSD). Enrichment factor for both thiamine and folic acid were in the ranges of 1088 and 905. The results are listed in **Table 4.**

TABLE 4: STATISTICAL PARAMETERS AND FIGURES OF MERIT FOR DETERMINATION OF ANALYTES IN HUMAN URINE AND SERUM SAMPLES BY APPLYING UA-DLLME METHOD.

Samples	LOD	Dynamic range	SEN	SEL	RSD
	(ng mL^{-1})	(ng mL^{-1})	(ng mL^{-1})		(%)
Thiamine	3.07	1-1000	0.62	0.80	3.01
Folic acid	11.15	5-750	0.49	0.73	2.78

10. Application of the proposed method to real samples: To evaluate performance of the proposed method, determination of Vitamin thiamine and folic acid in human urine and serum samples was carried out under the optimized conditions that mentioned above. The results indicating the thiamine and folic acid concentrations are shown in **Fig.7.a-b,** respectively. The results obtained by applying the UA-DLLME-HPLC to real and spiked human urine and serum samples are summarized in **Table 5.** The results showed that satisfactory recoveries of Vitamins could be obtained using the recommended method. The data obtained by this method revealed the ability of this method in the determination thiamine and folic acid in human

urine and serum samples by UA-DLLME-HPLC after coupling with a BBD without considerable errors. The chromatograms two-dimensional thiamine and folic acid and chromatograms two-dimensional thiamine and folic acid were recorded between the wavelength of 200 and 300 nm. **Fig. 6 a-e. Fig.7 a-c** shows the typical chromatograms of the extracted Vitamins in real samples before and after spiking with different concentration of each Vitamins.

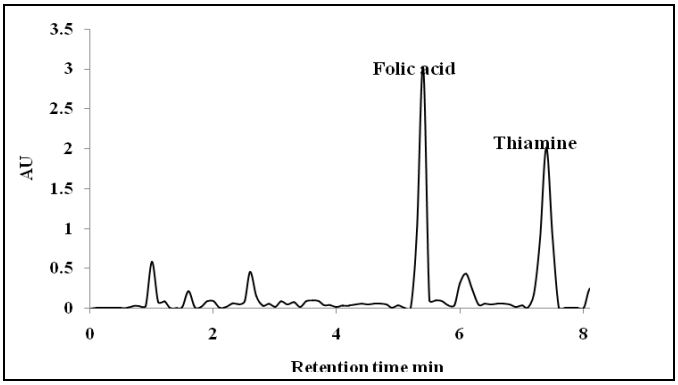


FIG.7.a

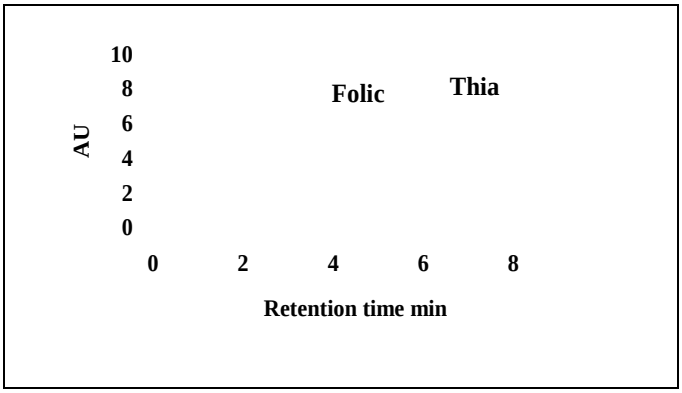


FIG.7.b

TABLE 5: ADDED AND FOUND VITAMINS CONCENTRATIONS (ng mL^{-1}) IN REAL HUMAN URINE AND SERUM SAMPLES (R1-R5)

Samples		Thiamine	Folic acid
R1	Added	0	0
	Found	10.0	25.0
	Recovery%		
R2	Added	2.0	5.0
	Found	11.15	30.81
	Recovery%	107.62	102.7
R3	Added	100.0	99.8
	Found	110.0	125.1
	Recovery%	100	100.24
R4	Added	0	0
	Found	9.6	24.9
	Rcovery%		
R5	Added	2.0	5.0
	Found	11.15	30.81
	Recovery%	106.62	102.7

CONCLUSION: A new method has been proposed for the simultaneous determination trace levels of thiamine and folic acid in human urine and serum samples using HPLC after optimization by UA-DLLME method. Advantages of this method include high recovery, simplicity of operation, low consumption of organic solvents, highly sensitive and selective procedure with good LODs. As the results, the UA-DLLME – HPLC coupled hybrid Box–Behnken design -genetic algorithm was successfully applied for the simultaneous *determination* thiamine and folic acid in human urine and serum samples.

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REFERENCES:

1. P.L Lynch, I.S Young. Determination of thiamine by high-performance liquid chromatography. J Chromatogr A 881(2000)267-284.
2. P. Vinas, C. Lopez-Erroz, N. Balsalobre, M. Hernandez-Cordoba. Reversed-phase liquid chromatography on an amide stationary phase for the determination of the B group Vitamins in baby foods. Journal of chromatography A, 1007(2003) 77-84.
3. Y. Mrestani, R.H Neubert. Thiamine analysis in biological media by capillary zone electrophoresis with a high-sensitivity cell. Journal of Chromatography A, 871(2000) 351-356.
4. P. L.M Lynch, I. S. Young. Determination of thiamine by high-performance liquid chromatography. Journal of Chromatography A, 881(2000) 267-284.
5. J. Wang, L. Ding, J. Hao, W. Zhao, Q. Zhou, M. Hao, Zhonghua yu fang yi xue za zhi, Interaction of folate deficiency and aberrant profile of DNA methyltransferase 1 in the progression of cervix carcinogenesis, Chinese journal of preventive medicine.46 (2012) 1088-1094.
6. M.V. Gamble, H. Ahsan, X. Liu, P. Factor-Litvak, V. Ilievski, V. Slavkovich, F. Parvez, J.H. Graziano, Folate and cobalamin deficiencies and hyperhomocysteinemia in Bangladesh, The American journal of clinical nutrition. 81 (2005) 1372-1377.
7. S. Zhao, H. Yuan, C. Xie, D. Xiao, Determination of folic acid by capillary electrophoresis with chemiluminescence detection, Journal of Chromatography A. 1107 (2006) 290-293.
8. C.R. Santhoshkumar, J.C. Deutsch, K.L. Hassell, N.M. Kolhouse, J.F. Kolhouse, Quantitation of red blood cell folates by stable isotope dilution gas chromatography-mass spectrometry utilizing a folate internal standard, Analytical biochemistry. 225 (1995) 1-9.
9. R. Hannisdal, P.M. Ueland, A. Svardal, Liquid chromatography–tandem mass spectrometry analysis of folate and folate catabolites in human serum, Clinical chemistry. 55 (2009) 1147-1154.

10. B. Álvarez-Sánchez, F. Priego-Capote, J.M. Mata-Granados, M.D. Luque de Castro, Automated determination of folate catabolites in human biofluids (urine, breast milk and serum) by on-line SPE-HILIC-MS/MS, Journal of Chromatography A. 1217 (2010) 4688-4695.
11. X.-H. Zheng, L.-Y. Jiang, L.-T. Zhao, Q.-Y. Zhang, L. Ding, Simultaneous quantitation of folic acid and 5-methyltetrahydrofolic acid in human plasma by HPLC–MS/MS and its application to a pharmacokinetic study, Journal of Pharmaceutical Analysis. 5 (2015) 269-275.
12. S.H. Kirsch, J.P. Knapp, W. olfgang Herrmann, Rima Obeid, Quantification of key folate forms in serum using stable-isotope dilution ultra performance liquid chromatography–tandem mass spectrometry, Journal of Chromatography. B 878(2009) 3865-70.
13. X.H. Zheng, L.Y. Jiang, L.T Zhao, Q. Ying Zhang, L. Ding, Simultaneous quantitation of folic acid and 5-methyltetrahydrofolic acid in human plasma by HPLC–MS/MS and its application to a pharmacokinetic study, Journal of Pharmaceutical Analysis 5(2015) 269-275.
14. J.E. Oosterink, E.F.G. Naninck, A. Korosi, P.J. Lucassen, J.B. van Goudoever, H. Schierbeek, Accurate measurement of the essential micronutrients methionine, homocysteine,Vitamins B6, B12, B9 and their metabolites in plasma,brain and maternal milk of mice using LC/MS ion trap analysis, Journal of Chromatography B. 998–999 (2015) 106-113.
15. M. Rezaee, Y. Assadi, M.-R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, Determination of organic compounds in water using dispersive liquid–liquid microextraction, Journal of Chromatography A. 1116 (2006) 1-9.
16. C.K. Zacharis, P.D. Tzanavaras, K. Roubos, K. Dhima, Solvent-based de-emulsification dispersive liquid–liquid microextraction combined with gas chromatography–mass spectrometry for determination of trace organochlorine pesticides in environmental water samples, Journal of Chromatography A. 1217 (2010) 5896-5900.
17. H. Chen, J. Ying, H. Chen, J. Huang, L. Liao, LC determination of chloramphenicol in honey using dispersive liquid–liquid microextraction. Chromatographia. 68 (2008) 629-634.
18. M.D. Luque de Castro, F. Priego-Capote, Ultrasound-assisted preparation of liquid samples, Talanta. 72 (2007) 321-334.
19. M. Ghaedi, H. Mazaheri, S. Khodadoust, S. Hajati, M.K. Purkait, Application of central composite design for simultaneous removal of methylene blue and Pb^{2+} ions by walnut wood activated carbon, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 135 (2015) 479-490.
20. N. Chamkouri, A. Niazi, V. Zare-Shahabadi, Development of a novel pH sensor based upon Janus Green B immobilized on triacetyl cellulose membrane: Experimental design and optimization, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 156 (2016) 105-111.
21. R. Leardi, Genetic algorithms in feature selection, in: J. Devillers (Ed.), In Genetic Algorithms in Molecular Modeling, Academic Press, London, 1996.
22. R. Leardi, R. Boggia, M. Terrile, Genetic algorithms as a strategy for feature selection, J. Chemom. 6 (1992) 267–281.

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