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Application of Non-bilinear Voltammetric Data for the First-Order Excellence in Simultaneous Determination of Ascorbic Acid, Uric Acid, Acetaminophen and Noradrenalin in Interfering Media

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Abstract
For the first time, an analytical methodology based ondifferential pulse voltammetry (DPV) at a glassy carbon electrode (GCE) assisted by two multivariate calibration (MVC) models including back propagation-artificial neural network (BP-ANN), non-linear class, and partial least squares-1 (PLS-1), classical class, thatthey have been constructed on the basis of non-bilinear first order differential pulse voltammetry (DPV) data,was developed and validated for the simultaneous determination of Ascorbic acid, Uric acid, Acetaminophen, and Noradrenalinto identify which approach offers the best predictions.The baselines of the DPV signals were corrected by asymmetric least square spline regression (AsLSSR) algorithm. Before applying the PLS-1,lack of bi-linearity was tackled by potential shift correction using correlation optimised warping (COW) algorithm. The multivariate calibration (MVC) model was developed as a quaternary calibration modelin a blank human serum sample (drug-free) provided by a healthy volunteer to regard the presence of a strong matrix effect which may be caused by the possible interferents present in the serum, and it was validated and tested with two independent sets of analytes mixtures in the blank and actual human serum samples, respectively.According to the obtained results, the PLS-1 was recommended for simultaneous determination of AA, UA, AC, and NA in both blank and actual human serum samples .

Keywords
Multivariate Calibration;Ascorbic Acid; Uric aAcid; Acetaminophen;Noradrenalin; Simultaneous Determination.

1. INTRODUCTION

Ascorbic acid (AA), Uric acid (UA), Acetaminophen (AC) and Noradrenalin (NA) usually can coexist in biological matrixes and they were investigated as main molecules for biological processes in human metabolism. For example, Ascorbic acid (AA, vitamin C), a water-soluble compound, generally exists in many fruits and plants [1]. It is also can prevent free radical-induced diseases, such as cancer and Parkinson's disease, through the reaction with free radicals [2]. Uric acid (UA) is another important biomolecule in human body, which is the primary product of purine metabolism [3].Abnormal concentration level of UA may cause such diseases as hyperuricaemia, gout and the Lesch–Nyan disease [4].Acetaminophen (AC) is a widely used analgesic and antipyretic drug [5]. Overdoses of acetaminophen produce toxic metabolites accumulation in liver, which may cause severe andsometimes fatal hepatoxicity [6,7] and nephrotoxicity [8].Noradrenalin (NA) is a neurotransmitter belonging to a catecholamine type hormone [9] that is manufactured as a drug. Alsocalled norepinephrine, especially by those in the medical field [10].

Many methods such as high performance liquid chromatography (HPLC) [11,12], gas chromatography [13], capillary zone electrophoresis [14],ion chromatography [15], biological fluids including titrimetry [16], UV–Vis spectrophotometry [17,18], have been reportedfor the determination of mentioned analyte.Nevertheless, above methodsare usually time-consuming, complicated and partly expensive. But electrochemical methods have gained much attention because of their quick response, high sensitivity, high accuracy, simple operation mode, low cost as well as ability to miniaturize.Their similar structural patterns and electrochemical signals, show overlapping when using conventional electrodes. Therefore, it is important to develop new approaches for the simultaneous determinations of these drugs. An attractive way is the use of chemometric and multivariate calibration methods.

One problem which restricted the application of chemometrics in electroanalytical chemistry is the non-linearity of electrochemical data [19]. According to the literature, the shift in electrochemical responses can be originated from adsorptive phenomena on the electrode surface, pH variations in the cell or fluctuations in the composition of cell solution [20]. However, this situation has been scarcely described for electrochemical signals. Whereaszeroth-order univariate calibration cannot detect sample components producing an interfering signal, first-

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order MVC, which operates using a vector of data per sample, may compensate for these potential interferents, provided they are included in the calibration set,a property known as the "first-order advantage" [21]. The MVC methods are increasingly used to extract relevant information from different types of absorptive spectral and electrochemical data,which have maximum information regarding the analyte of interest while discards those carrying irrelevant information (noise, saturation regions) or those heavily overlapped with other sample components which are not of interest, to predict analyte concentrations or properties of complex samples [22–24]. A basic assumption for application of linear MVC models is the data bilinearity, which may be compromised by the above commented potential shifts. Therefore several strategies have been developed for the calibration of non-linear data systems such as: a) data pretreatment or data alignment; for this reason, data alignment was performed before applying PLS-1, b)the use of linear methods (for slight nonlinearities only), c) the use of local modeling, d) the addition of extra variables, e) the use of non-linear calibration techniques [25-27] and the most important linear calibration method is PLS [28].Mathematical pre-processing techniques exist for removing variations in voltammograms from run to run, which are unrelated to analyte concentration changes [29,30]. The removal of these unwanted effects leads to more parsimonious PLS models and often produce better statistical indicators. Three type of MVC models were usually used such as: classical linear (PLS-1, CPR, and MLR), robust linear (PRM, and RCR) and non- linear (PLY-PLS, SPL-PLS, RBF-PLS, LS-SVM, WT-ANN, DWT-ANN, and BP-ANN) that we choose PLS-1 (from classical class) and BP-ANN (from non-linear class) methods for determining the concentration of the studied analytes.

In this work, we present the development of an electroanalytical methodology based on DPVat a GCE and comparison of two chemometric approachesincluding classical linear (PLS-1)and non- linear (BP-ANN)MVC models for the simultaneous determination of four studied analytesin human serum samples which have a very complex matrix.Literature survey revealed that no attempt has been made till date to the simultaneous voltammetric determination of AA, UA, AC, and NA with the aid of Chemometrics.

2. EXPERIMENTAL

2.1. Chemicals and solutions

AA, UA, AC, and NAwere purchased from Sigma-Aldrich.A phosphate buffered solution (PBS, 0.1mol L^{-1}) of pH2.0was prepared from chemicals (analytical grade) including NaH_2PO_4 and $Na_2HPO_4$from Merck.All other chemicals used in the investigation were of analytical grade obtained from regular sources and used without further purification.Stock standard solutions of AA, UA, AC, and NA were prepared by exact weighing and dissolving of their solid powder in distilled water (10.0 mL) with a concentration level of 0.1 mol $L^{-1}$and were stored at dark in a refrigerator until analysis time. Working solutions were prepared by appropriate dilution of the stock standard solutions with PBS (0.1mol L^{-1}, pH 2.0). All the solutions were prepared by doubly distilled water (DDW).

2.2. Apparatus and softwares

Electrochemical experiments were performed using anAutolab(Eco Chemie BV, Netherlands) controlled by the NOVA software (Version 1.8). A conventional three-electrode cell was used with a saturated Ag/AgCl as reference electrode, a Pt wire as counter electrode and a GCE as working electrode. The pH of the solutions was adjusted using aJENWAY-3510pH meter equipped by a combined glass electrode.All the recorded electrochemical data was smoothed, when necessary, and converted to data matrices by the use of several home-made m-files in MATLAB environment (Version 7.14, MathWorks, Inc.). All the computations for baseline correction, data alignment and multivariate calibration (MVC) were performed in MATLAB environment.All the computations were performed on a DELL XPS laptop (L502X) with Intel Core i7-2630QM 2.0GHz, 8 GB of RAM and Windows 7-64 as its operating system.

2.3. Preparation of theserum samples

A blank human serum sample (drug-free) was provided by a healthy volunteer who not exposed to any drug for at least 10.0 months. An actual human serum sample was collected from apatient under AC treatmentwhich kindly provided by a Medical Diagnostic Laboratory in Kermanshah, Iran. The following methodology was used to prepare all the serum samples: according to the method of Shu et al. [31], to eliminate protein and other substances, 5.0 mL of human serum sample was placed in a10.0 mL glass tube and 5.0 mL of 15.0 % (w/v) Zinc Sulfate solution-Acetonitrile (50/40,v/v) was added. The glass tube was vortexed for 20.0 min, maintained at 4.0 °C for 15.0 min followed by centrifugation at 4000.0 rpm for 5.0 min. Then, the supernatant was collected in the same tube and this solution was used for subsequent analyses.

2.4. Model efficiency estimation

Whether a model can be applied to analysis of human serum samples or not, model validation is

possibly the most important step in the model building sequence. In order to evaluate the performance of MVC models, each model was validated for the prediction of validation and test sets, evaluating root mean square errors of prediction (RMSEP), and relative error of prediction (REP).

$$RMSEP = \sqrt{\frac{\sum_{1}^{n}\left(y_{pred} - y_{act}\right)^2}{n}} \quad (1)$$

$$REP(\%) = \frac{100}{y_{mean}}\sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_{pred} - y_{act})^2} \quad (2)$$

where y_{act} and y_{pred} are actual and predicted concentrations of each component, respectively, and*n*is the number of samples in validationor test set.

2.5. Electrochemical procedure

Prior to electrochemical experiments, the GCE was successively polished to a mirror using 0.3 and 0.05 µm Alumina slurry. Afterward, the electrode was washed thoroughly with ethanol and DDW and dried at room temperature. All electrochemical experiments were carried out at room temperature. The DPV measurements were carried out at the following operating conditions for the four studied analytes: step potential 0.005 V, modulation amplitude 0.025 V, modulation time 0.05 s,interval time 0.5 s, and scan rate 0.05 V s^{-1}.

3. RESULT AND DISCUSSION

3.1. Electrochemical studies

3.1.1. pH dependencestudy

To select the best pH for the simultaneous determination of AA, UA, AC, and NA, the effect of pH on the peak current of the cyclic voltammograms of AA, UA, AC, and NA was investigated. Fig. 1A-D shows the influence of the pH of the PBS (0.1mol L^{-1}), in the range of 2.0-10.0, on the signal intensities of 0.1 mM AA, UA, AC, and NA. As can be observed in Fig.1 A-D, all peak currents of the studied analytes have a maximum value at pH 2.0. Taking into account that for analytical purposes both maximal and stable currents are necessary, a pH value of 2.0 was selected for further experiments. The oxidation peak potential of all studied analytes shifted to less positive values as the pH of the buffer solution was increased (Fig. 1A-D).

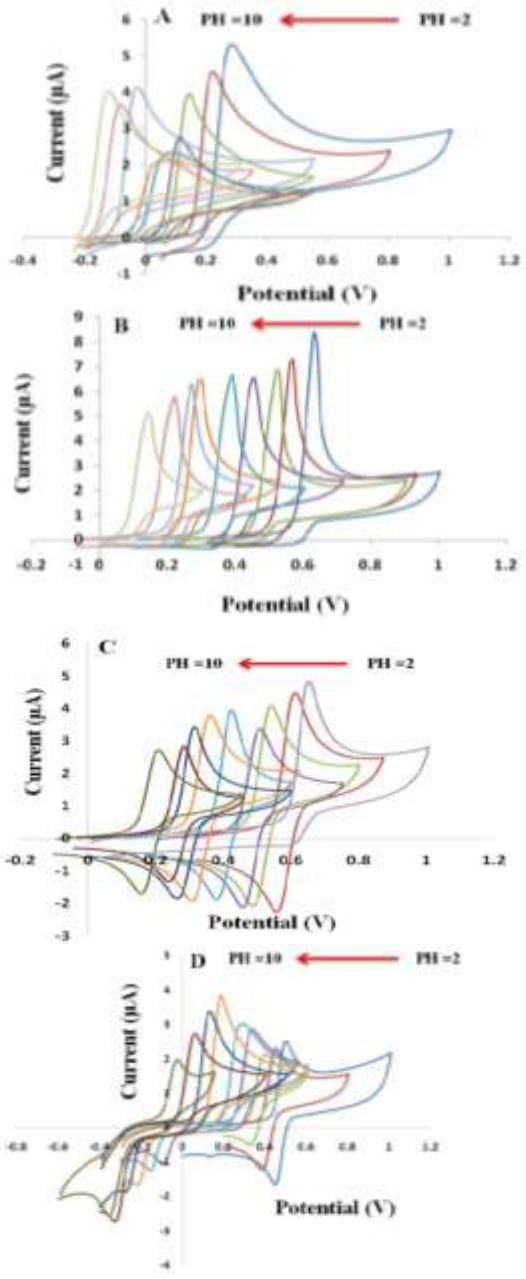

Fig. 1. Cyclic voltammograms of (A) AA (0.1×10^{-3} mol L^{-1}), (B) UA (0.1×10^{-3} mol L^{-1}), (C) AC (0.1×10^{-3} mol L^{-1}), and (D) NA (0.1×10^{-3}mol L^{-1}), in 0.1mol L^{-1} PBS at different pHs.

3.1.2. Effect of scan rate

The influences of scan rate (υ) on the peak current (I_P) of AA, UA, AC, and NA at the GCE in PBS (0.1mol L^{-1}, pH 2.0) were studied by cyclic voltammetry. Fig. 2 A-D shows the influence of the scan rate (υ) on the peak current (I_P) of 0.1 mM AA, UA, AC, and NA at the GCE in PBS

(0.1 mol L^{-1}, pH 2.0). In the range of 10.0-1000.0 mV s^{-1}, a linear relationship was established between I_P and$\upsilon^{1/2}$, for all of the studied analytes, indicating the diffusion controlled mechanism except UA.The linear regression equations are$I_{p,AA}$(µA)=3.7828+27.486$\upsilon^{1/2}$V s^{-1}(R^2=0.9920), $I_{p,UA}$(µA)=-2.683+45.517υV s^{-1}(R^2= 0.9823),$I_{p,AC}$(µA)=0.5868-0.2653$\upsilon^{1/2}$ V s^{-1} (R^2= 0.9914) and$I_{p,NA}$(µA)=-0.5157+8.8232$\upsilon^{1/2}$V s^{-1}(R^2= 0.9980).

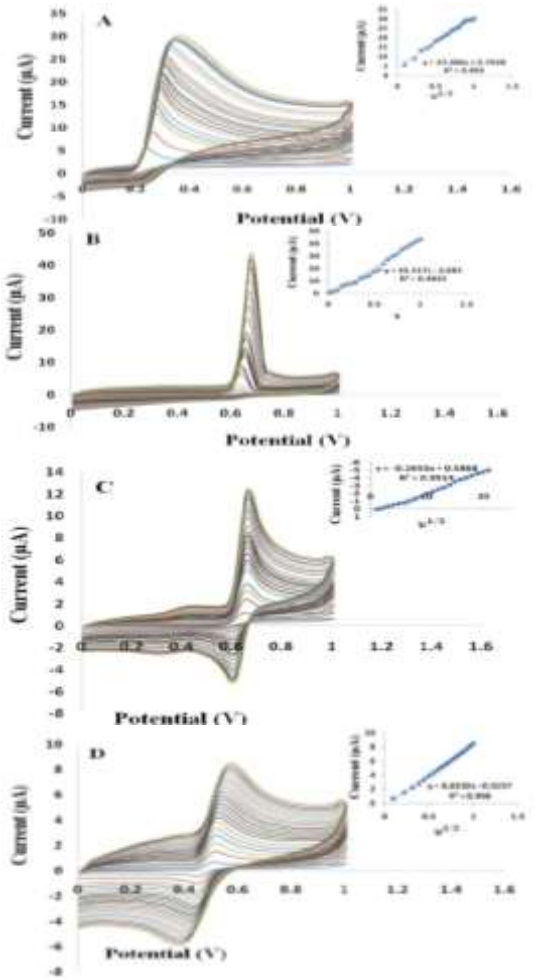

Fig. 2. Cyclic voltammograms of (A) AA (0.1×10^{-3} mol L^{-1}), (B) UA (0.1×10^{-3} mol L^{-1}), (C) AC (0.1×10^{-3} mol L^{-1}), and (D) NA (0.1×10^{-3} mol L^{-1}), in 0.1 mol L^{-1} PBS (0.1 mol L^{-1}, pH 2.0) at different scan rate.Insets: dependence of I_p with scan rate.

3.2. Chemometric studies

3.2.1. A glance to necessity of MVC

Fig. 3shows the cyclic voltammograms of AA (curve a), UA (curve b), AC (curve c), NA (curve d), and their mixture (curve e) in PBS (0.1mol L^{-1}, pH 2.0). In all conditions evaluated, a strong signal overlapping was observed for the simultaneous analysis of AA, UA, AC, and NA at the GCE (see Fig. 3, curvee). Thus, the quantification of any of these analytes will be biased if univariate calibration is used as analytical method, and for tackling this problem it was necessary to use MVC.

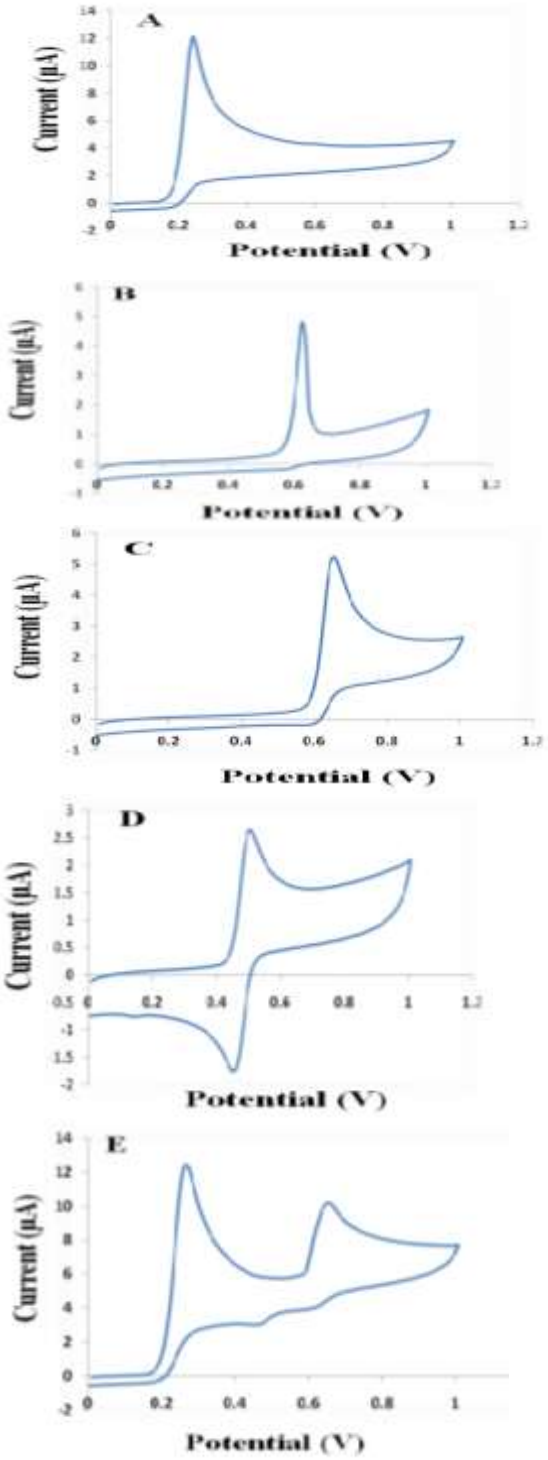

Fig. 3. Cyclic voltammograms of AA (curve a), UA (curve b), AC (curve c), NA (curve d), and their mixture (curve e) in PBS (0.1 mol L^{-1}, pH 2.0).

3.2.2. Calibrations

3.2.2.1. Univariate calibrations

Prior to multivariate calibration experiments, univariate calibration experiments were performed (Fig. 4A-D) and calibration curves were constructed with several points as peak current versus analyte concentration in the range10.0 to 360.0 $\times 10^{-6}$ mol $L^{-1}$for AA (inset of Fig. 4A), 5.0to 370.0$\times 10^{-6}$ mol $L^{-1}$for UA (inset of Fig. 4B), 1.0 to 80.0$\times 10^{-6}$mol L^{-1} for AC (inset of Fig. 4C), 3.0 to 150.0$\times 10^{-6}$ mol $L^{-1}$for NA (inset of Fig. 4D), and evaluated by linear regressionwhich were the limiting assayed concentrations in subsequent analyses. All analytes showed linear dependences between peak current and concentration at different concentrations intervals.

3.2.2.2. Multivariate calibrations

When the analytes are analyzed in the presence of interferences, the electrochemical profile revealed additional changes to those observed in the absence of interferences. The main changes observed were minor alterations in the baseline and displacement of peak potential, probably due to modification in viscosity of the solution and consequently the diffusion coefficient of the analytes. This effect produces alterations in the chemometrics responses and for this reason, the calibration and validation sets were prepared in a blank human serum sample (drug-free).

3.2.2.2.1. Calibration set

The human serum has a complex matrix and may contain a lot of unexpected interferences therefore, if the presence of these interferences was not considered during calibration, a first-order MVC model would give biased predictions of the concentration of the analytes of interest. Therefore, the calibration set was prepared in a blank human serum sample (drug-free) which was collected from a healthy volunteer to regard the complex matrix of the serum sample which may contain a lot of unexpected interferences. This strategy was applied in order to provide PLS-1 and BP-ANNenough information concerning the signals of the analytes when they are embedded into the real background. All the calibration mixtures (the compositions of the calibration mixtures were selected according to a central composite design (CCD), (Table 1) were prepared in the blank human serum sample spiked with an appropriate amount of each analyte of interest considering the linear calibration ranges (previously established from univariate calibrations for each analyte).All samples were diluted with PBS (0.1 mol L^{-1}, pH 2.0) to adjust the pH and then appropriate amounts of these diluted samples were transferred to the electrochemical cell, and the solutions were measured in random order.Final concentration of each analyte was obtained by multiplying the detected value by the appropriate dilution factor.

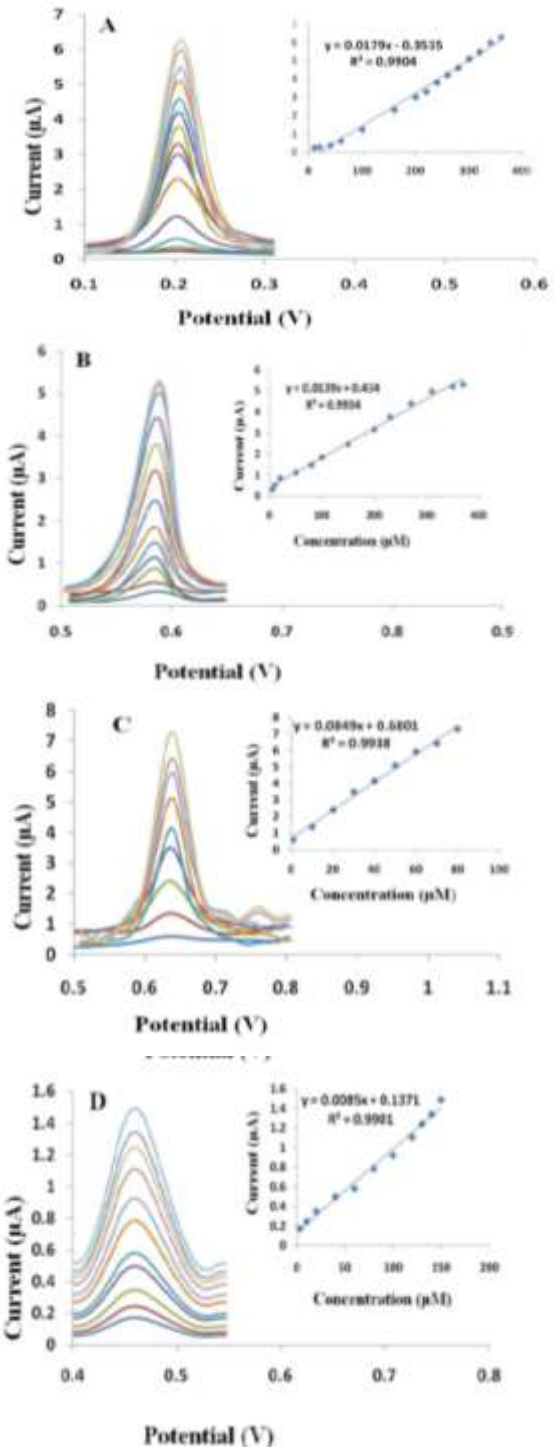

Fig. 4. Representative differential pulse voltammograms of (A) AA, (B) UA, (C) AC, and (D) NA,PBS (0.1 mol L^{-1}, pH 2.0)at different concentrations. Insets: dependence of I_p with concentration.

Table 1. Concentration data of calibration set (**C_1-C_{30}**), validation set (**V_1-V_{10}**), and test set (**T_1-T_{10}**).

Sample	AA (10^{-6} mol L^{-1})	UA (10^{-6} mol L^{-1})	AC (10^{-6} mol L^{-1})	NA (10^{-6} mol L^{-1})	Sample	AA (10^{-6} mol L^{-1})	UA (10^{-6} mol L^{-1})	AC (10^{-6} mol L^{-1})	NA (10^{-6} mol L^{-1})
C_1	97.5	96.25	20.75	39.75	V_1	20	12	4	5
C_2	97.5	96.25	20.75	113.25	V_2	45	32	55	16
C_3	97.5	96.25	60.25	39.75	V_3	32	76	33	72
C_4	97.5	96.25	60.25	113.25	V_4	17	11	78	33
C_5	97.5	278.75	20.75	39.75	V_5	54	55	11	133
C_6	97.5	278.75	20.75	113.25	V_6	90	340	15	144
C_7	97.5	278.75	60.25	39.75	V_7	250	233	22	99
C_8	97.5	278.75	60.25	113.25	V_8	345	187	76	43
C_9	272.5	96.25	20.75	39.75	V_9	52	121	47	21
C_{10}	272.5	96.25	20.75	113.25	V_{10}	131	88	66	6
C_{11}	272.5	96.25	60.25	39.75	T_1	78	15	32	12
C_{12}	272.5	96.25	60.25	113.25	T_2	23	22	6	55
C_{13}	272.5	278.75	20.75	39.75	T_3	311	333	77	43
C_{14}	272.5	278.75	20.75	113.25	T_4	45	345	54	143
C_{15}	272.5	278.75	60.25	39.75	T_5	66	132	33	22
C_{16}	272.5	278.75	60.25	113.25	T_6	22	11	11	76
C_{17}	10	187.5	40.5	76.5	T_7	187	17	65	54
C_{18}	360	187.5	40.5	76.5	T_8	243	65	39	57
C_{19}	185	5	40.5	76.5	T_9	11	54	28	88
C_{20}	185	370	40.5	76.5	T_{10}	82	33	18	91
C_{21}	185	187.5	1	76.5					
C_{22}	185	187.5	80	76.5					
C_{23}	185	187.5	40.5	3					
C_{24}	185	187.5	40.5	150					
C_{25}	185	187.5	40.5	76.5					
C_{26}	185	187.5	40.5	76.5					
C_{27}	185	187.5	40.5	76.5					
C_{28}	185	187.5	40.5	76.5					
C_{29}	185	187.5	40.5	76.5					
C_{30}	185	187.5	40.5	76.5					

3.2.2.2.2.Validation set

To check the prediction ability of the model after optimizing all calibration parameters, avalidation set of ten quaternary mixtures (Table 1) was prepared in the blank human serum sample (drug-free).The concentrations of four analytes were selected at random from the corresponding calibration ranges. All samples were diluted with PBS (0.1 mol L^{-1}, pH 2.0) to adjust the pH and then appropriate amounts of these diluted samples were transferred to the electrochemical cell, and the solutions were measured in random order. Final concentration of each analyte was obtained by multiplying the detected value by the appropriate dilution factor.

3.2.2.2.3. Test set

With the purpose of evaluating the proposed method in a very interfering environment such as human serum, atest set of tenquaternary mixtures (Table 1) was prepared in the actual serum (see Section 2.3.)with random amount of each analyte of interest in the same concentration range used for calibration. All samples were diluted with PBS (0.1 mol L^{-1}, pH 2.0) to adjust the pH and then appropriate amounts of these diluted samples were transferred to the electrochemical cell, and the solutions were measured in random order. Final concentration of each analyte was obtained by multiplying the detected value by the appropriate dilution factor.It should be noted that all the samples related to the test set were prepared in the actual serum (see Section 2.3) which contains AC therefore, the exact concentrations of the spiked AC were computed by a previous knowledge about the initial amount of AC inthe serum which was obtained by analyzing the serum with HPLC-UV method prior to analyzing by the proposed method in this study.

3.2.3. Pretreatment and data arrangement

Besides the problem arising from the presence of severely overlapping analyte profiles, in the present study two additional complications may occur: (1) interactions among analytes and the background interferents present in the serum, which may cause signal changes in comparison with pure analyte profiles, and (2) sample-to-

sample potential shifts in the analyte profiles, which are common in voltammetric studies.For tackling the first problem, it was necessary to include the possible interferents in the calibration set in order to allow a first order algorithm to model the analyte-background interactions before prediction on new samples. Concerning the second commented problem, some preprocessing alternatives were independently applied on the electrochemical responses before model building and validation [21,32,33,34].

In our previous work [21] we pointed out that voltammetric performance can be enhanced by eliminating noise and background components therefore, baseline elimination is a crucial step for reducing both complexity and number of the unexpected components. Moreover, it was demonstrated that the use of signal pre-treatments such as baseline- and potential shift-corrections improve the quality of first-order voltammetric signals and, as a consequence, the performance of resolution by first-order algorithms [21]. In the next sections, some strategies areexaminedto achieving the mentioned aims.

3.2.3.1. Baseline correction

Baseline correction has been considered as a critical step for enhancing the signals and reducing the complexity of the analytical data [35]. Considering this aim, we used the method proposed by Eilers et al. [36] for background elimination in two-dimensional signals based on asymmetric least squares splines regression approach. In the asymmetric least squares method [37] the following cost function is minimized:

$$Q=\sum_i \upsilon_i (y_i-f_i)^2+\lambda\sum_i (\Delta^2 f_i)^2 \quad (3)$$

where*y* is the experimental signal, *f* is a smooth approximation of baseline trend (*y*), Δ is the derivative of *f*, i denotes successive values of the signal, the positive parameter *λ*is a regularization parameter that weigh the second term and *υ*are weights. The positive deviations from the estimated baseline (peaks) have low*υ* values while the negative deviations (baseline) obtain high*υ* values. In the multidimensional extension of baseline correction method, Eilers et al. proposed the splines-based approach to smoothing instrumental signal (the penalty term in Eq. 1). Details of the implementation of the mentioned method can be found in the literature [36].

3.2.3.2. Potential shift correction

For chemometric model building, several strategies have been proposed to align shifted signals such as chromatograms, electropherograms or NIR spectra. One of the most popular ones is correlation optimised warping (COW) [38,39]. However, this situation has been scarcely described for electrochemical signals [21]. According to the literature, the shift in electrochemical responses can be originated from adsorptive phenomena on the electrode surface, pH variations in the cell or fluctuations in the composition of cell solution, among others [20].

A basic assumption for application of a multivariate calibration model is the data bi-linearity, which may be compromised by the above commented potential shifts. Therefore, the DPV signals were aligned towards a target signal using COW. The COW algorithm was introduced by Nielsen et al. [38] as a method to correct for shifts in discrete data signals. It is a piecewise or segmented data preprocessing technique that uses dynamic programming to align a sample signal towards a reference signal by stretching or compression of sample segments using linear interpolation. First, the segment and slack were optimized using a simplex-like optimization routine and then mean voltammogram was selected as target“signal”.

The results of baseline and shift corrections are shown in Fig. 5. Fig. 5A-C shows the raw DPV data recorded for the calibration, validation and test sets, respectively. Fig. 5D-Fshow the results of baseline-corrected data of calibration, validation and test sets, respectively, and as can be seen the baselinesare satisfactorily corrected. Fig. 5G-I show the results of applying COW for data alignment and it confirms the capability of COW for aligning the data. The baseline- and shift-corrected data was used to model building by PLS-1 while the baseline-corrected data without shift-correction was used to model building by BP-ANN.

3.3. Performance evaluation of PLS-1 and BP-ANN in blank and actual serum samples

In MVC calibration, it is usual to have two data sets: a calibration set, employed to build the regression model, and a validation set to check the prediction ability of the model after all calibration parameters have been optimized, but in this study we are going to determine the analytes’ concentrations in actual human serum samples which have a very complex matrix due to the presence of many interferents present in the serum therefore, with the purpose of evaluating the method in the presence of interferents, a test set was also prepared in anactual human serum sample.

Before calibration with PLS-1, it is usual to assess the optimum number of latent variables in order to avoid overfitting, by applying the well-known cross-validation method described by Haaland and Thomas [40].With the purpose of estimating the number of optimum latent variables for ACO-GA-SS-PLS-1 leave-one-sample-out cross-validation was performed.

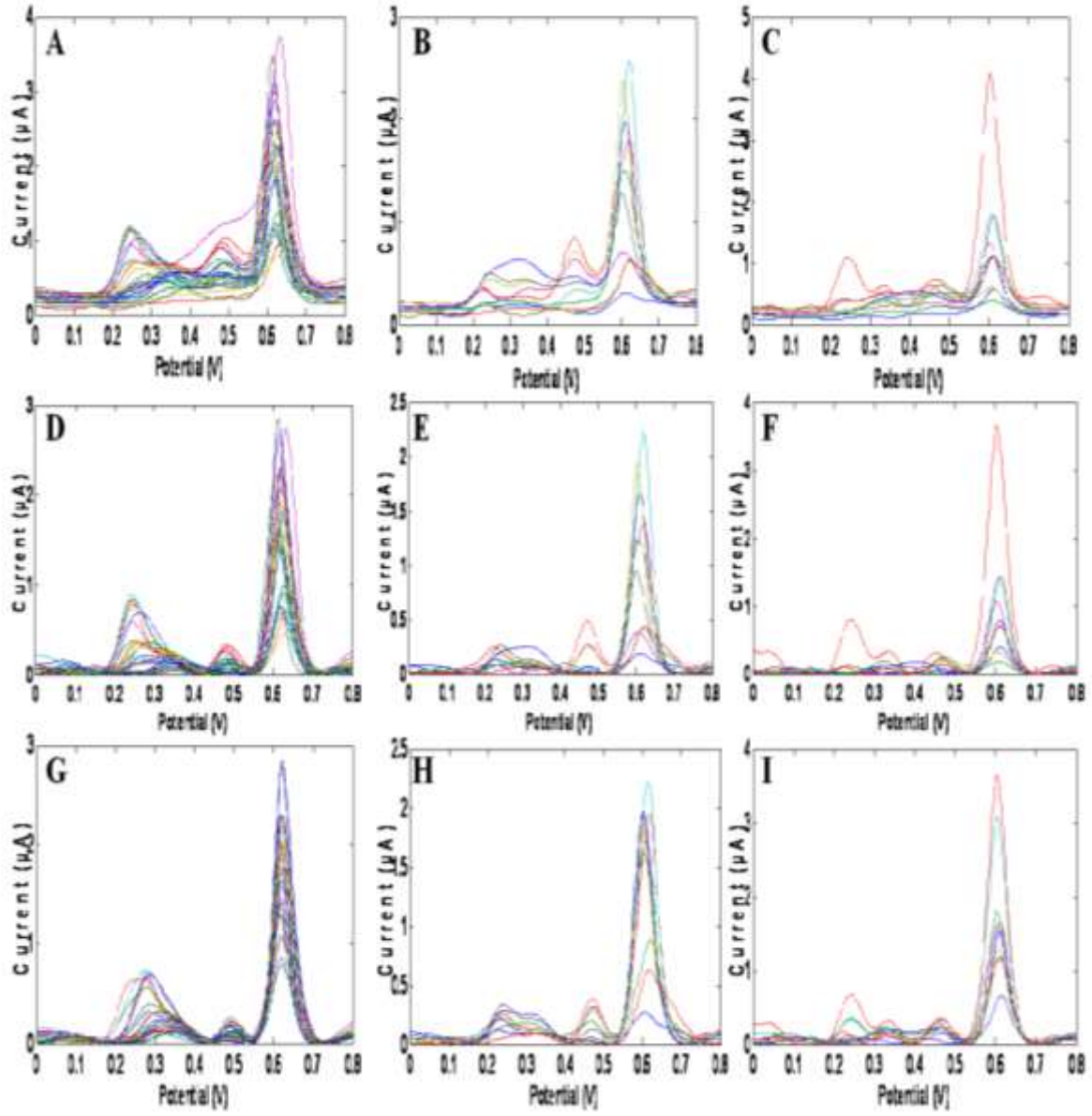

Fig. 5. (A)-(C): Raw data related to calibration, validation, and test sets, respectively, (D)-(F):Baseline corrected data by AsLSSR related to calibration, validation, and test sets, respectively,and (G)-(I): shift corrected data by COW related to calibration, validation, and test sets, respectively.

The optimum number of factors is estimated by calculating the ratios F (*A*) = PRESS (*A*<*A**)/PRESS (*A*), where PRESS =Σ ($c_{i,act}$-$c_{i,pred}$)^2, *A* is a trial number of factors, *A** corresponds to the minimum PRESS, and $c_{i,act}$and $c_{i,pred}$ are the actual and predicted concentrations for the *i*th sample left out of the calibration during cross validation, respectively. Then, the number of factors leading to a probability of less than 75% that *F*> 1 is selected.This analysis led to the conclusion that the latter number is 4 for all the cases, as expected for this system using a mean centering procedure. The presence of non-linearities which are usually found in electrochemical data causes more latent variables to model the variability of the data but in the present study this limitation was tackled by potential shift correction.Then, the PLS-1 algorithm was run on the calibration, and validationsets.According to the results presented in Table 2,with very satisfactory values for RMSEPs and REPs for the four analytes of interest it is apparent that the PLS-1 approach has found the correct answer.

Table 2. Results of application of PLS-1 and BP-ANN to validation and test sets.

	PLS-1				BP-ANN			
Validation set	AA	UA	AC	NA	AA	UA	AC	NA
V_1	19.8	11.9	4.03	5.02	18.85	12.98	4.5	5.4
V_2	45.3	32	55.9	15.99	47.1	34.6	57.3	17.1
V_3	31.8	76	33.6	72.03	34.3	73.2	31.2	74.9
V_4	16.92	11.01	79.2	33.1	18.9	10	76.5	35.6
V_5	54.9	55.1	11.3	133	55.4	52.1	10.2	137.9
V_6	90.1	340.1	14.9	144	92.3	349.9	14.1	152.6
V_7	250.3	234.1	21.6	99.1	252.3	238.7	20.1	104.5
V_8	345.4	188.1	76.5	43.01	346.7	190.9	73.2	47.8
V_9	52.6	120.1	47.9	21.01	54.9	126.4	49.6	24.3
V_{10}	132.03	87.5	65.8	6.02	138.5	90.8	68.9	5.02
RMSEP ()	0.5164	0.5924	0.6301	0.0469	3.0777	4.5571	1.9773	4.2385
REP ()	0.4985	0.5129	1.5482	0.0820	2.9707	3.9456	4.8584	7.4099
	PLS-1				BP-ANN			
Test set	AA	UA	AC	NA	AA	UA	AC	NA
T_1	78.2	15.2	32.4	11.9	80.1	16.3	30.1	10.2
T_2	23.2	22.3	5.9	55.07	21.1	24.3	7.1	58.8
T_3	311.8	333.6	77.3	42.9	307.3	339.8	75.2	46.3
T_4	44.2	345.3	53.5	142.9	43.1	349.9	42.3	140.1
T_5	65.1	132.1	32.6	21.9	64.2	138.9	31.2	19.3
T_6	21.7	11.2	10.8	75.9	20.1	13.2	9.9	79.9
T_7	188.5	17.2	64.3	53.2	190.8	19.4	63.2	51.1
T_8	244.1	65.6	38.5	56.4	249.6	62.3	37.8	53.2
T_9	11.4	53.2	27.8	87.8	12.1	52.1	26.2	91.2
T_{10}	82.4	32.4	17.66	90.8	85.9	31.1	16.9	88.8
RMSEP ()	0.7771	0.4505	0.4007	0.3368	3.2648	3.8723	3.9815	3.1210
REP ()	0.7276	0.4387	1.1038	0.5255	3.0569	3.7705	10.9685	4.8609

Table 3. ANN training results for all data sets.

AA	Value
Architecture (input-hidden-output neurons)	2000-05-01
Number of training epoch	450-1200
Learning rate	0.5
Momentum	0.5
UA	Value
Architecture (input-hidden-output neurons)	2005-04-01
Number of training epoch	400-8735
Learning rate	0.5
Momentum	0.5
AC	Value
Architecture (input-hidden-output neurons)	2000-06-01
Number of training epoch	450-10080
Learning rate	0.5
Momentum	0.5
NA	Value
Architecture (input-hidden-output neurons)	2004-06-01
Number of training epoch	600-9765
Learning rate	0.5
Momentum	0.5

With the purpose of analyzing the potentiality of the evaluated methodology based on DPV data processed by PLS-1, a test set of ten quaternary mixtures (see Table 1) was prepared in an actual serum (see Section 3.2.2.2.3.).According to the results presented in Table 2 for the test set, with very satisfactory values for RMSEP and REP for the four analytes of interest it is apparent that the PLS-1 approach has found the correct answer.
Intrinsically non-linear data can be processed with non-linear methods. Therefore BP-ANN was also used to predict the validation and test sets using the training parameters reported in Table 3. According to the results presented in Table 2 for both validation and test sets, satisfactory values for RMSEP and REP for the four analytes of interest were not founded and it shows that the BP-ANN approach has not found the correct answer.

Therefore, in this study PLS-1 shows better results than BP-ANN therefore,for the sake of a further investigation into the accuracy of the proposed method (PLS-1), thepredictedconcentrations of both validation and test sets were regressed on the nominal concentrations. In this case an ordinary least squares (OLS) analysis of predicted concentrations versus nominal concentrations was applied [41]. The calculated intercept and slope were compared with their theoretically expected values (intercept = 0, slope = 1), based on the elliptical joint confidence region (EJCR) test. If the ellipses contain the values 0 and 1 for intercept and slope (ideal point), respectively, showing the predicted and nominal values do not present significant difference at the level of 95% confidence and the elliptic size denotes precision of the analytical method, smaller size corresponds to higher precision [42]. Fig. 6A-Dand Fig. 6F-I show the regression of predicted concentrations on nominal values based on OLS method corresponding to the validation and test sets, respectively, and Fig. 6E and Fig. 6J show the corresponding ellipses of the EJCR analyses for validation and test sets, respectively.As can be concluded from Fig. 6A-D and Fig. 6F-I, the predictions for AA, UA, AC, and NA in both validation and test sets are in good agreement with the nominal values. If the EJCRs for validation set are analyzed (Fig. 6E), it is notable that while the ellipses for AA, UA, and NA include the theoretically expected point (ideal point), indicating accuracy of the developed methodology for these analytes, the ideal point falls on the AC ellipse (red ellipse), denoting slightly poorer prediction accuracy for AC. But all the ellipses in the test set contain the ideal point (Fig. 6J) which shows the accurate determination of all analytes in test set by the developed methodology.The statistical results shown in Table 2, with adequate values for RMSEPand REP for all analytes, do also support this conclusion.

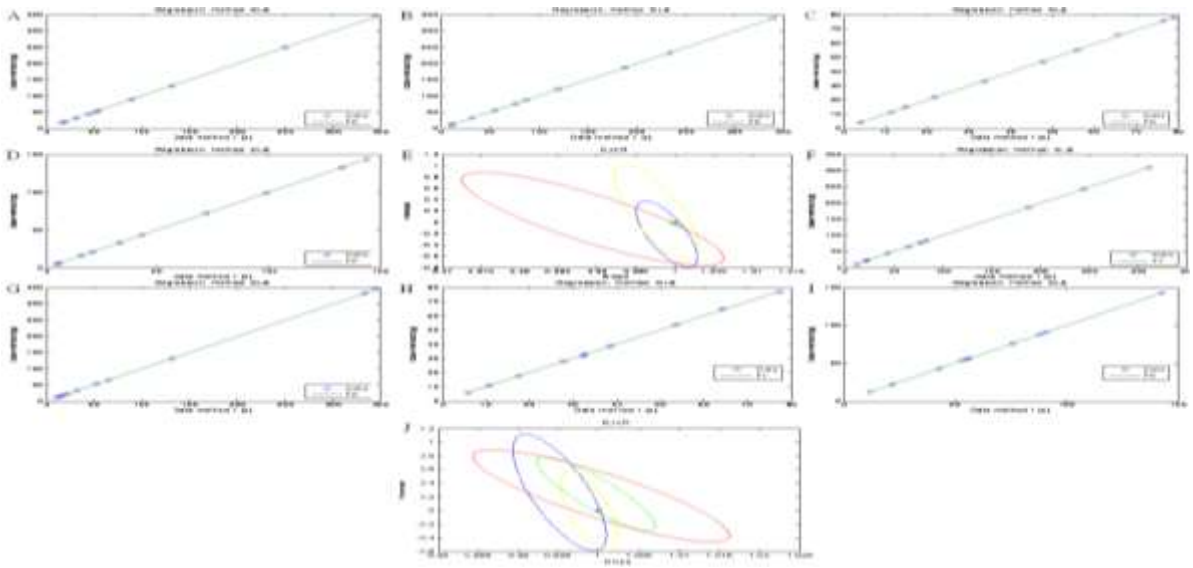

Fig. 6. (A)-(D) and (F)-(I): plots for predicted concentrations as a function of nominal values for AA, UA, AC, and NAin validation and test set, respectively. (E) and (J):elliptical joint regions (at 95% confidence level) for the slopes and intercepts of the regressions for AA (blue ellipse), UA (yellow ellipse), AC (red ellipse), and NA (green ellipse) in validation and test set, respectively. Plus point(+) marks the theoretical (0,1) point.

4. CONCLUSION

In the present article, combination of electrochemistry with chemometrics led us to introduce an efficient analytical method for simultaneous determination of AA, UA, AC, and NA at a GCE in very interfering media. The four studied analytes exhibited a strong voltammetric overlapping for the simultaneous analysis of these compounds. The overlapping was more successfully resolved using PLS-1 with preprocessing than BP-ANN. The baseline of the DPV signals was successfully removed by an efficient chemometric algorithm. Because of the non-bilinear behavior of the experimental data, the potential shift correction was carried out by COW as an efficient chemometric algorithm before applying PLS-1.To regard the presence of a strong matrix effect which may be caused by the possible interferents present in the human serum sample, the MVC models were built and validated in a blank human serum sample (drug-free) provided by a healthy volunteer which allowed us to exploiting first-order advantage for the simultaneous determination of the four studied analytes in very interfering media such as human serum samples. Finally, the application of the developed method based on PLS-1 to simultaneously assay the concentrations of AA, UA, AC, and NA in an actual human serum sampleallowed to obtain satisfactory results. This study allows one to propose the present method as a promissory, cheap and accessible alternative for routine determination of the concentrations of AA, UA, AC, and NA in human serum samples.

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کاربرد داده های ولتامتری غیر دو خطی به دلیل برتری روش های مرتبه اول در اندازه گیری همزمان آسکوربیک اسید، اوریک اسید، استامینوفن و نورآدرنالین در محیط تداخل

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چکیده

یک روش تجزیه ای بر اساس ولتامتری پالس تفاضلی در سطح الکترود کربن شیشه‌ای با همکاری دو روش چند متغیره کمومتریکسی شامل پس انتشار- شبکه عصبی مصنوعی که از روشهای غیر خطی می باشد و روش حداقل مربعات جزئی ۱- که از روش‌های کلاسیک است و در هر دو روش از داده های ولتامتری پالس تفاضلی که قاعدتاً دادهای مرتبه اول غیر خطی هستند استفاده گردیده است، برای اندازه‌گیری همزمان اسکوربیک اسید، اوریک اسید، استامینوفن و نورآدرنالین توسعه و اعتبار سنجی می شوند تا مشخص گردد که کدام روش پیش بینی بهتری را برای غلظت گونه های یاد شده ارائه می دهد. خط زمینه طیف‌های ولتامتری پالس تفاضلی توسط الگوریتم رگرسیون کوچکترین مربعات نامتقارن تصحیح گردید. جابجایی پتانسیل و مشکل دوخطی بودن داده‌ها، پیش از استفاده از روش حداقل مربعات جزئی ۱- با به کارگیری الگوریتم همبستگی انحراف بهینه شده اصلاح شد. مدل کالیبراسیون چند متغیره به عنوان یک مدل کالیبراسیون چهارتایی در یک نمونه سرم انسانی خالی (بدون دارو) ارائه شده توسط یک داوطلب سالم برای در نظر گرفتن وجود یک اثر ماتریکس قوی که ممکن است توسط تداخل‌های احتمالی موجود در سرم ایجاد شود، ایجاد شد و با دو مجموعه مستقل از مخلوط آنالیت‌ها در نمونه‌های خالی و واقعی سرم انسانی اعتبارسنجی و آزمایش شد. در نهایت، روش حداقل مربعات جزئی ۱- برای اندازه گیری همزمان همزمان اسکوربیک اسید، اوریک اسید، استامینوفن و نورآدرنالین هم در نمونه سرم انسانی شاهد و هم در نمونه سرم انسانی مورد بررسی نتایج بهتری را در مقایسه با پس انتشار- شبکه عصبی مصنوعی نشان داد.

واژه‌های کلیدی

روش چند متغیره؛ اسکوربیک اسید، اوریک اسید؛ استامینوفن و نورآدرنالین؛ اندازه‌گیری همزمان.