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Full Paper

Simultaneous Determination of Caffeine, Theobromine, and Theophylline in Tea by Differential Pulse Adsorptive Stripping Voltammetry Combined with Chemometrics

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Abstract- Adsorptive stripping voltammetry (AdSV) using a glassy carbon electrode combined with chemometrics was investigated for simultaneous determination of caffeine, theobromine, and theophylline in tea samples, offering high selectivity, sensitivity, simplicity, and cost-effectiveness. The optimal electrochemical conditions were 0.01 mol.L^{-1} H_2SO_4, an adsorption potential of 0.6V *vs.* Ag/AgCl/KCl, a scan rate of 0.025 V/s, and an adsorption time of 60 s. Linear calibration graphs for each compound were obtained in the concentration ranges from 1.0×10^{-6} to 4.0×10^{-5} mol.L^{-1}, 1.0×10^{-6} to 3.0×10^{-5} mol.L^{-1}, and 1.0×10^{-6} to 1.4×10^{-5} mol.L^{-1} for caffeine, theobromine, theophylline, respectively. In this study, a pre-separation step was not required despite the overlapping voltammetric peaks of caffeine, theobromine, and theophylline in the mixtures, as chemometrics techniques, such as partial least squares (PLS), principal component regression (PCR), and classical least squares (CLS) were applied. Among the three multivariate linear regressions, the PLS method was chosen because it has the smallest relative error, all less than ±11.1%. In contrast, the CLS performed poorly with relative reaching up to ±83%. The proposed novel method was applied to simultaneously determine caffeine, theobromine and theophylline in tea samples. The results showed no significant differences compared to those obtained using high-performance liquid chromatography (HPLC).

Keywords- Adsorptive stripping voltammetry; Chemometrics; Caffeine; Theobromine; Theophylline; Tea samples

1. INTRODUCTION

Caffeine (1,3,7-trimethylxanthine), theobromine (3,7-dimethylxanthine), and theophylline (1,3-dimethylxanthine) (Figure 1) are methylxanthine derivatives commonly used in the pharmaceutical industry as therapeutic agents with anti-depressant, anti-inflammatory as well as for stimulating gastric secretion, the central nervous system and acting as a diuretic. Naturally, they can be found in tea, coffee, chocolate, and products made from these substances [1].

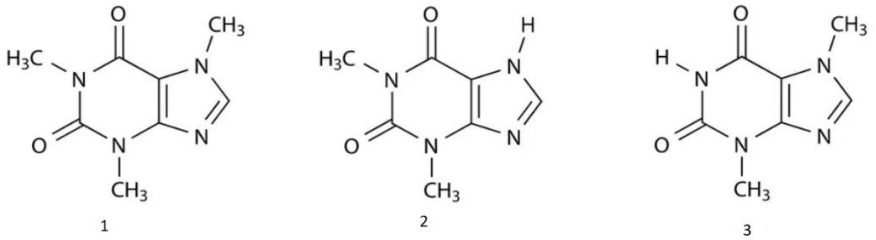

Figure 1. Chemical structure of three methylxanthines: 1) Caffeine; 2) Theophylline; 3) Theobromine

Despite the potential negative effect of caffeine, theobromine and theophylline on human health. They are widely consumed in large amounts through daily products such as coffee, tea, soft drinks, pharmaceutics [2]. Therefore, selective and sensitive methods for their simultaneous determination need to be developed and improved to enable quick and accurate analysis.

To date, for the analysis of caffeine, theobromine, and theophylline in real samples, among the commonly used instrumental analytical methods, the HPLC-UV technique has consistently been the first choice due to its significant potential for simultaneous quantification and low limit of detection (LOD) but a long time analysis required [3-11]. Spectrophotometry, on the other hand, offers simple and rapid analysis, but without the support of chemometrics or machine learning [12,13], it can only determine caffeine, theobromine, and theophylline individually due to their spectral overlap [14]. Voltammetry, when using traditional electrodes like glassy carbon, can also only determine one of the three compounds at a time due to similar peak appearances on the voltammogram [15]. Therefore, the researchers' efforts have focused on electrode modification to simultaneously determine two or three of these compounds, employing materials such as boron-doped diamond (BDD) [16,17] with a linear concentration range of 1- 400×10^{-6} mol.L^{-1}, mesoporous carbon/nafion modified electrodes [18,19] with the detection limits of TP and CF were 0.37×10^{-6} mol.L^{-1} and 0.47×10^{-6} mol.L^{-1} (S/ N = 3) with linear ranges of 0.8×10^{-6} mol.L^{-1}-180.0×10^{-6} mol.L^{-1} and 1.3×10^{-6} mol.L^{-1}-230.0×10^{-6} mol. L^{-1}, respectively, graphene/nafion composite film modified glassy carbon electrode [20] was used for the simultaneous determination of paracetamol, aspirin, and caffeine (CAF).

Even though the methodologies mentioned above are highly efficient for simultaneous determining the concentration of individual compounds in the mixture, the overlaping peak potentials of caffeine, theobromine, and theophylline in the presence of large amounts of the compounds is still a noticeable obstacle to be concerned. To address this, several solutions have been proposed, including simplifying the complex analytical data by using pre-separation steps to give the specific reactions to the compounds of interest. Additionally, the chemometrics data-driven approaches offer a powerful strategy to extract the I–E matrix from the mixture voltammograms to deduce the contribution of the target compound in the mixture with the addition of multiple linear regression (MLR), including principal component regression (PCR) and partial least squares (PLS) compare with classical least square method [21].

In this study, trace amounts of caffeine, theobromine, and theophylline in aqueous extracts of green tea samples were simultaneously analyzed using AdsV combined with multivariate linear regression algorithms. This approach involved constructing a calibration matrix containing all three analytes and a signal matrix of peak current values at different adsorption potentials. The accuracy of the model was evaluated, and the method was applied to analyze real samples

2. EXPERIMENTAL SECTION

2.1. Reagents and instrumentation

All chemicals and reagents used in this study were of analytical grade. Throughout the experiments, double-distilled water was employed. Caffeine, theobromine, theophylline, and H_2SO_4 were purchased from Sigma-Aldrich, Germany.

Stock solution of caffeine 10^{-2} mol.L^{-1}, theobromine 10^{-2} mol.L^{-1}, theophylline 10^{-2} mol.L^{-1} was prepared in double distilled water. Working standard solutions containing all three analytes were subsequently prepared in 0.06 M sulfuric acid (H_2SO_4).

The AdSV experiments were conducted in a µAutolab type III (Netherlands), connected to a 663 -VA electrode assembly (Metrohm, Switzerland), and operated through 757 VA software. All experiments were conducted using a standard three-electrode configuration, comprising a bare glassy carbon electrode (GCE, 3 mm in diameter) as the working electrode, a carbon rod as the auxiliary electrode, and a silver/silver chloride electrode (Ag/AgCl, saturated KCl) as the reference electrode.

High performance liquid chromatography (HPLC) measurements were conducted on a Shimadzu LC-20 A system equipped with a DAD detector (set at 271 nm). A Lichrospher C-18 reverse phase (5 µm×250 mm×4.6 mm) column was utilized. The mobile phase consisted of 85% buffer (potassium phosphate, pH= 3.0) and 15% acetonitrile with a flow rate of 1.2 mL.min^{-1}.

2.2. Preparation of working electrode

The surface of the glassy carbon electrode (GCE) which is prone to contamination by other substances, and may reduce the repeatability of the method, required proper cleaning and activation. To prepare the working electrode, the GCE electrode was polished with aluminum oxide powder (size 1 µm) on a velvet towel until it became glossy, smooth, and mirror-like. To make sure that all remaining alumina particles were removed from the GCE's surface, it was thoroughly rinsed three times with double-distilled water before being ultrasonically cleaned in the same water to ensure the removal of any residual alumina particles from its surface. Then, the electrode was treated and activated by cyclic scanning from -0.6 V to 1.8 V in 0.1 $mol.L^{-1}$ H_2SO_4 until a stable cyclic voltammogram was obtained. It was performed about 10 cycles with scan rate 0.25 V/s. After that, the electrode was rinsed with double distilled water again.

2.3. Preparation of tea samples

Weigh approximately 1 gram of raw tea samples and soak it in 40.0 mL of boiling water for 10 minutes. Decant the mixture five times, then transfer the entire extracted solution to a 250.0 mL volumetric flask. Fill the flask to accurate volume with double distilled water. Next, dilute 1.0 mL of the extracted sample solution to 50.0 mL using 1.5 mL of 1 $mol.L^{-1}$ sulfuric acid and double distilled water.

The prepared solution was transferred into an electrochemical cell and purged with nitrogen gas for 5 minutes to remove any oxygen dissolved and measured under the optimal conditions. The multivariate linear regressions, a mathematical model to capture the relationship between peak current and concentration was used for the determination of caffeine (CAF), theobromine (TB) and theophylline (TP) in tea samples.

2.4. Determination of analytes using Differential Pulse Adsorptive Stripping Voltammetry (DPAdSV)

There are two steps involved in the Differential Pulse Adsorptive Stripping Voltammetry (DPAdSV) study of CAF, TB, and TP:

Stage 1: CAF, TB, and TP are adsorbed onto the working electrode's surface (GCE) at the selected adsorption potential (-0.2 V) in an open circuit state (no current in the measurement system) for a certain enrichment time. This process involves stirring the solution at a speed of 300 rpm.

Stage 2: The potential in the circuit is scanned anodically from E_1 = 0.8 V to E_2 = 1.6 V in order to obtain the oxidation peaks of three analytes adsorbed on the electrode surface.

For the peaks separated on the voltammogram, the peak current intensity obtained is directly proportional to the analyte concentrations in the sample. In the case of peak overlap,

the intensity versus potential matrix over the scanned potential range was used in multiple linear regression with the concentration matrix.

3. RESULTS AND DISCUSSION

3.1. Study on electrochemical properties of CAF, TB and TP

The electrochemical behaviors of each CAF, TB and TP were investigated using the cyclic voltammetry (CV) under the following conditions: concentration of 10^{-5} mol.L^{-1} CAF, TB, TP, 0.1 mol.L^{-1} H_2SO_4, a scan rate of 100 mV.s^{-1}, and potential scanning range from 0.5 V to 1.8 V. The cyclic voltammograms were recorded in two cases: (1) without adsorption and (2) with adsorption at 0.5 V for 60 s.

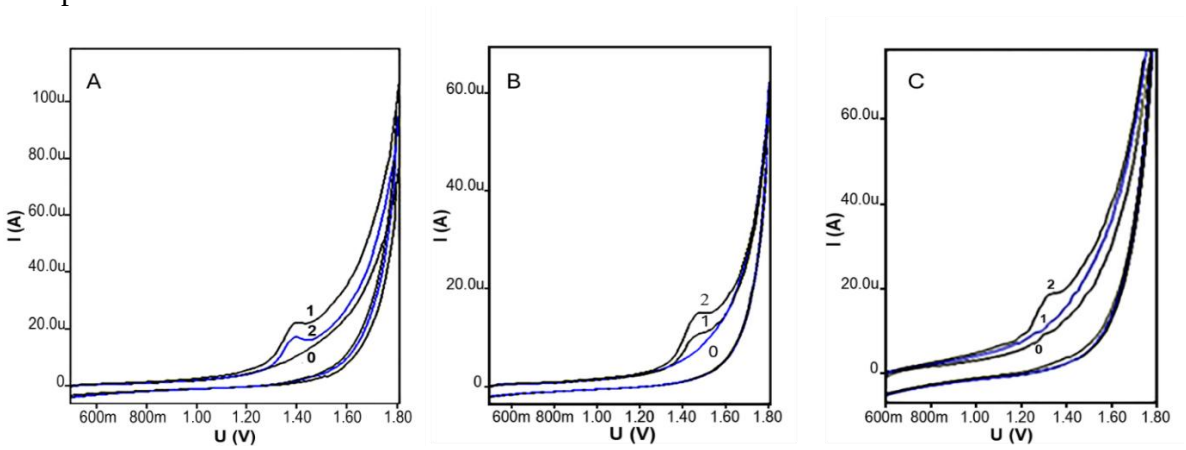

Figure 2. Cyclic voltammograms of A) CAF, B) TB, C) TP. 0) blank sample 1) 10^{-5} mol.L^{-1} CAF solution without adsorption t_{ad} = 0 s; 2) 10^{-5} mol.L^{-1} CAF at E_{ad} = 0.5 V with adsorption time 60 s

Figure 2 (A, B, C) shows that a single oxidation peak was observed at 1.4 V for CAF; at 1.42 V for TB; and as a peak shoulder at 1.28 V for TP. However, no reduction peak was detected in the reverse potential scan. The electrochemical process that took place on the active glassy carbon electrode's surface was therefore irreversible. The results suggest that the overlap among the three potential peaks caused the hard work to distinguish individual signals.

To investigate the adsorption behavior of CAF, TB, and TP on the surface of the active glassy carbon electrode, the cyclic voltammograms were recorded under varying adsorption time 0 s, 60 s and longer duration whereas the other were kept constant as adsorption potential of 0.5 V of 10^{-5} mol.L^{-1} CAF, TB, TP in 0.1 mol.L^{-1} H_2SO_4 solution. The results demonstrated that the oxidation potential peak of CAF, TB, TP depended on the adsorption time. Specifically, when the adsorption time was 60 s (curve 2), the oxidation peak current was higher than that without adsorption (curve 1) indicating that CAF, TB, and TP adsorbed onto the electrode surface. However, longer absorptions for all samples were performed but there was no improvement for the voltammetric signals.

CAF, TB, and TP in a mixture the electrochemical behavior of a mixture of 10^{-5} mol.L^{-1} CAF, TB, TP in 0.1 mol.L^{-1} H_2SO_4 was investigated simultaneously using CV at a scan rate of 100 mV.s^{-1} (Figure 3). The oxidation processes of CAF, TB, TP at the activated glassy carbon electrode were found to be irreversible and adsorptive on the electrode surface. However, only two oxidation peak potentials were observed, due to the overlap of one peak potential. Practically, the oxidation peak at 1.28 V corresponded to TP, while the peak at 1.45 V represented a mixture of CAF and TB. The peak potential of the mixture of CAF and TB was shifted compared to the individual peak potential of CAF and TB. This indicates that, the AdSV combined with chemometrics should be used to determine simultaneously.

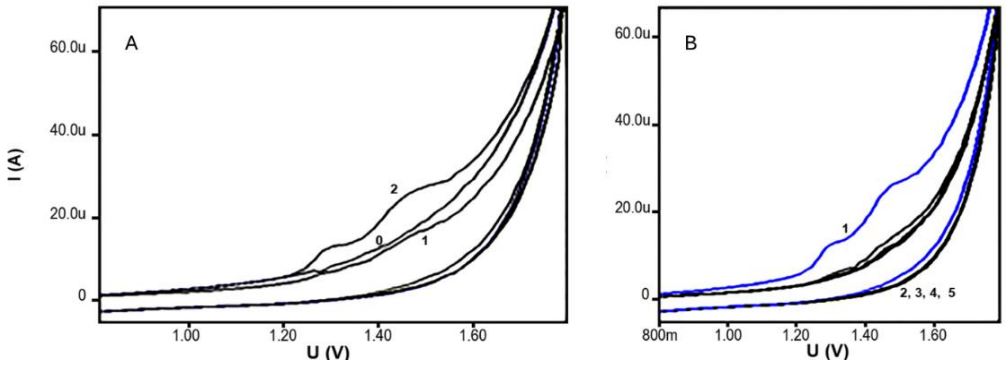

Figure 3. A) Cyclic voltammograms for a mixture of 10^{-5} mol L^{-1} CAF, TB, and TP at without adsorption t_{ad} = 0 s (1) and after adsorption 50 s at 0.5 V (2) in 0.1 M H_2SO_4 at scan rate 100 mV.s^{-1}; B) Cyclic voltammograms for a mixture of 10^{-5} mol L^{-1} CAF, TB, and TP, (1) after adsorption 50 s at 0.5 V, (2,3,4,5) repetitive cycle of (1, 2, 3, 4) at the same glassy carbon electrode surface in 0.1 M H_2SO_4 at scan rate 100 mV.s^{-1}

As shown in Figure 4, the anodic peak current decreased rapidly successive cycles (curves 2, 3, 4, and 5) compared to the first anodic cycle. This decrease suggests that CAF, TB and TP are desorbed from the glassy carbon electrode surface. The peak current in repetitive cycles 2, 3, 4, 5 was very low and similar to the signal observed in the blank sample. This indicates that the mixture of CAF, TB, and TP dissolved completely into the solution after the first cycle, resulting in no significant peak current being observed in subsequent cyclic voltammograms. These findings suggest that caffeine, theobromine and theophylline form monolayer adsorption on the surface of activate glassy carbon electrode.

3.2. Optimization of the experimental conditions for CAF, TP, TB determination by differential pulse adsorptive stripping voltammetry (DPAdSV)

3.2.1. The effect of the H_2SO_4 concentration

pH plays a critical role in electroanalytical chemistry as it influences the redox behavior of electroactive molecules. From the literature review, in this study H_2SO_4 was selected as the

supporting electrolyte to determine CAF, TB, and TP in tea samples. The effect of concentration of H_2SO_4 was investigated within a range of 0.005 mol.L^{-1} to 0.5 mol.L^{-1} under the following conditions: concentrations of CF, TB, and TP were all 10^{-5} mol.L^{-1}, adsorption potential was set at 0.6 V, adsorption time was 60 s, scanning rate was 0.025V/s. The results were shown in Figure 5, demonstrating how varying H_2SO_4 concentrations affected the oxidation peak currents and redox behavior.

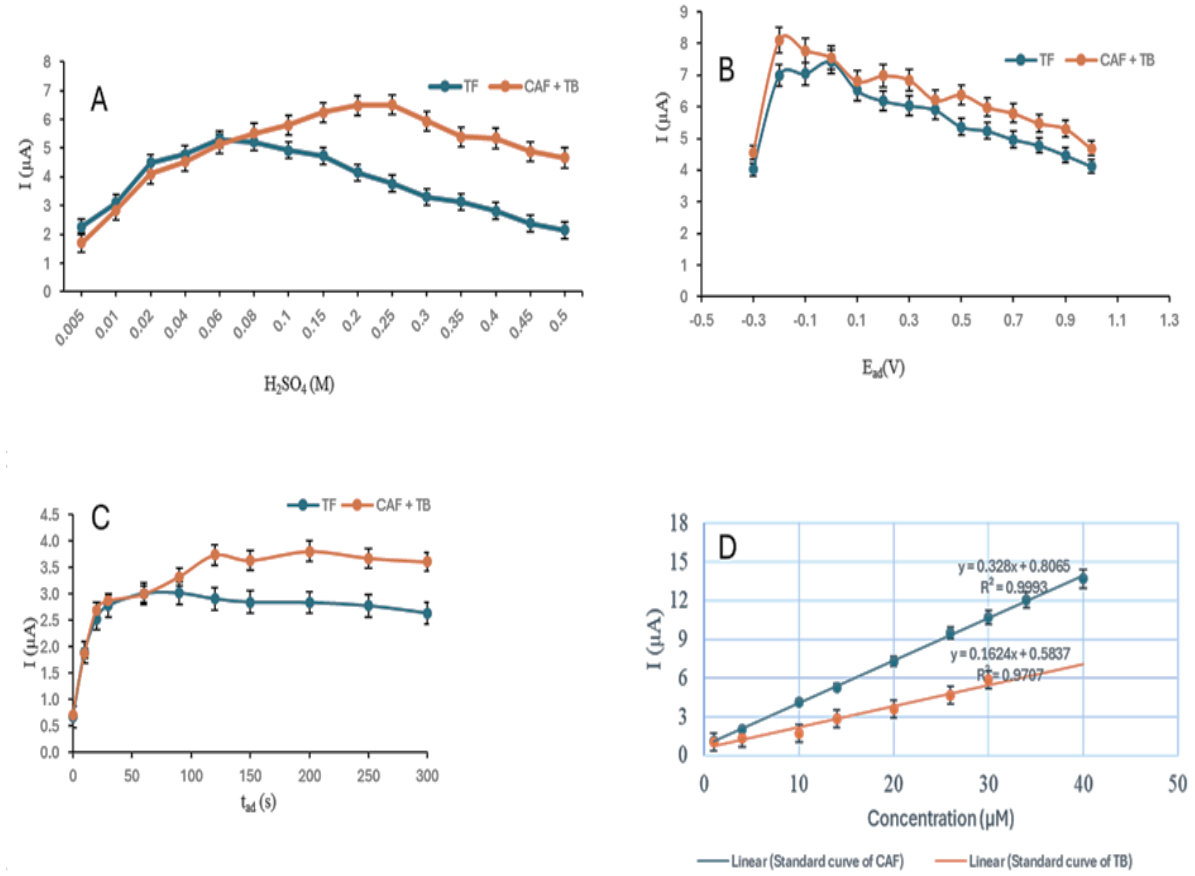

Figure 4. Optimization of the experimental conditions for CAF, TP, and TB determination: A) Effect of H_2SO_4 concentration on the anodic stripping peak current of 10^{-5} mol.L^{-1} CAF, TB, and TP at E_{ad} = 0.5 V, t_{ad} = 30 s, scan rate 0.04 V s^{-1}; B) Effect of adsorption potential on the anodic stripping peak current of 10^{-5} mol.L^{-1} CAF, TB, TP at different adsorption potential, t_{ad} = 30 s, scan rate 0.04 V s^{-1}; C) The effect of adsorption time to peak current; D) Standard curve of CAF (10^{-6} mol.L^{-1} to 4×10^{-5} mol.L^{-1}) and TB (1.0×10^{-6} mol.L^{-1} to 3.0×10^{-5}mol.L^{-1})

According to the results shown in Figure 4A, when the concentration of H_2SO_4 increases from 0.005 mol.L^{-1} to 0.06 mol.L^{-1}, the oxidation peak current of TP and CAF, TB also increased. The highest peak current for TP 5.31µA was observed at H_2SO_4 0.06 mol.L^{-1}. When the concentration of H_2SO_4 was further increased to 0.5 mol.L^{-1}, the oxidation peak current of TB and CAF continue to rise with the maximum peak current of 6.51µA recorded at H_2SO_4

0.25 mol.L^{-1}. However, the peak current of TP began to decrease when H_2SO_4 concentration reached 0.06 mol.L^{-1} or more. Additionally, at higher H_2SO_4 concentrations, the two oxidation peak potentials shifted toward to the more positive value, causing the peak potential of TP to become closer to those of CAF and TB. As a result, H_2SO_4 at a concentration of 0.06 mol.L^{-1} was chosen to determine CAF, TB, and TP in samples for this experiment.

3.2.2. The effect of AdSV parameters

The sensitivity and selectivity of AdSV are influenced by the adsorption potential of the analyte on the surface of the working electrode. To investigate this, the dependence of the stripping peak current on the adsorption potential was studied over the range from -0.3 V to 1.0 V (*vs.* Ag/AgCl/KCl) for 10^{-5} mol.L^{-1} CAF, TB, and TP after an adsorption time of 30 s. The results showed that the peak current was slightly decreased when the adsorption potential exceeded -0.2 V (Figure 4B). This suggests that the adsorption efficiency of CAF, TB, and TP on the electrode surface diminishes at higher adsorption potentials, and an adsorption potential of -0.2 V was kept for the measurements.

The adsorption time of the analyte at the working electrode surface could also affect the sensitivity of the adsorptive stripping voltammetry procedure. To optimize this parameter experiments were conducted under the following conditions: 10^{-5} mol L^{-1} CAF, TB and TP in 0.06 M H_2SO_4 with an adsorption potential of 0.6 V, and adsorption time ranging from 0 to 30 s. Figure 4.C shows that the peak current increased rapidly during the first 30 seconds of adsorption, followed by a slight increase between 30 and 120 seconds, after which the peak current stabilized for longer adsorption periods. Based on these results, adsorption time of 30 s and 120 s were selected for tests involving higher and lower concentration than 10^{-5} mol L^{-1}, respectively. In general, the optimal differential pulse parameters for the determination of CAF, TB, and TP were established as follow: a scan rate of 25 mV.s^{-1}, a pulse amplitude of 50 mV, frequency 50 Hz.

3.2.3. Validation the developed method for determination of TP

Under the optimal conditions, the peak current was found to depend on the concentration of TP as show in Figure 5A. A linear calibration graph for TP in a mixture of 10.0×10^{-6} mol.L^{-1} CAF and 1.0×10^{-6} mol.L^{-1} TB was obtained with the concentration range from 1×10^{-6} mol.L^{-1} to 14×10^{-6} mol.L^{-1} (Figure 5B). The relationship between the peak current (I_p) and TP concentration (C_{TP}) was described by the linear equation $Ip = 0.46 \times C_{TP}$ (10^{-6} mol.L^{-1}) - 0.32 with $R^2 = 0.9997$, (n = 8) indicating a very good correlation between the peak current and TP concentration.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated using the equation LOD (or LOQ) = $k.S_D/b$, where k=3 for LOD and k=10 for LOQ, SD is the standard deviation of the regression and b is the slope of the calibration curve. The LOD and LOQ for TP were found to be 2.6×10^{-7} mol.L^{-1} and 8.6×10^{-7} mol.L^{-1}, respectively.

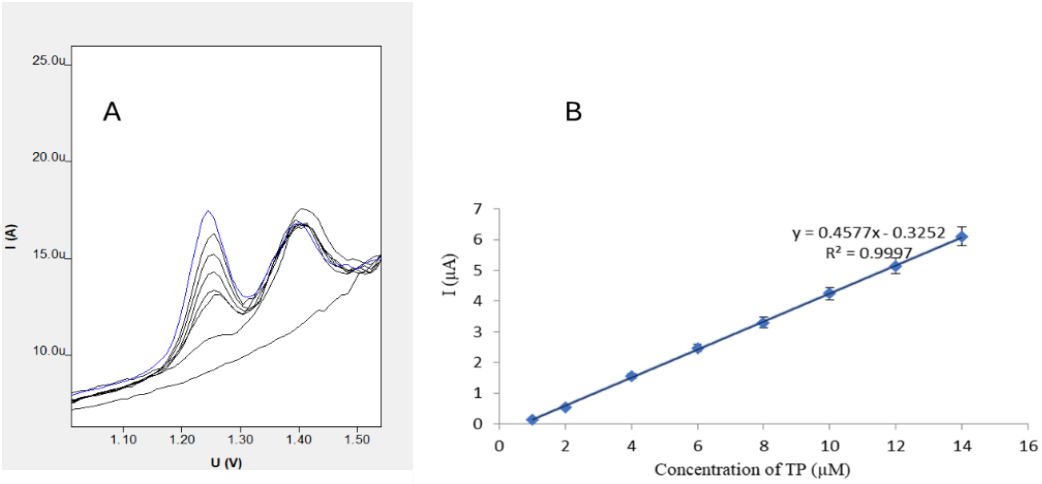

Figure 5. AdSV voltammograms (A) and Standard curve (B) depend on TP from 1.0×10^{-6} mol.L^{-1} to 14×10^{-6} mol.L^{-1} in the mixture of 10.0×10^{-6} mol.L^{-1} CAF and 1.0×10^{-6} mol.L^{-1} TB

Repeatability was evaluated by conducting ten replicate measurements for TP concentration of 1.2×10^{-5}, 2.8×10^{-6}, 7.2×10^{-6} mol.L^{-1} in the presence of 10^{-4} mol.L^{-1} CAF and 2.10^{-5} mol.L^{-1} TB in 0.1 mol.L^{-1} H_2SO_4 after 30 s preconcentration time at adsorption potential of -0.2 V. A relative standard deviation (RSD) of less than < 3 % (n=10) was achieved. The mean recovery of TF in tea sample ranged from 98.2% to 105.1%. The results indicated that the developed method had good repeatability and high accuracy.

3.2.4. Simultaneous determination of CAF, TP, and TB.

Due to the overlapping peak potentials of CAF and TB, direct quantification was challenging. Consequently, multivariate regression was employed to determine CAF, TP, and TB. Initially, calibration curves were constructed for each individual substance (Figure 4D). For CAF, the calibration curve was established within a concentration range of 1.0×10^{-6} to 4.0×10^{-5} mol.L^{-1} in 0.06 mol.L^{-1} H_2SO_4 with an adsorption time of 60 s, an adsorption potential of 0.6 V, and a scan rate of 25 mV.s^{-1}. A linear calibration curve for the CAF concentration in a range of 10^{-6} mol.L^{-1} to 4×10^{-5} mol.L^{-1} was obtained following the equation: Ip = 0.33×C (10^{-6} mol L^{-1}) + 0.81 with R^2 = 0.9993. The LOD and LOQ were determined to be 1.18×10^{-6} mol L^{-1} and 3.93×10^{-6} mol.L^{-1}, respectively. Similarly, a standard curve for TB was established within a concentration range of 10^{-6} to 3×10^{-5} mol.L^{-1}. The relationship between TB concentration and peak current was linear within the concentration range of 1.0×10^{-6} mol. L^{-1} to 30.0×10^{-6} mol.L^{-1}, following the equation Ip = 0.16× C_{TB} (10^{-6} mol. L^{-1}) + 0.58 with a R^2 = 0.994. The LOD and LOQ were 2.2×10^{-7} mol.L^{-1} and 7.3×10^{-7} mol.L^{-1}, respectively. The mean recovery of TB in tea samples ranged from 94.8% to 99.2%.

Linear multivariate regression methods (CLS, PLS, and PCR) were applied for the simultaneous determination of CAF and TB based on the least squares algorithm using the entire voltammogram. A regression model was constructed based on the concentration matrix

of 30 standard solutions containing CAF, TB, and TP within the investigated linear range (Table 1). Samples 1 to 23 were used for model development, while samples 24 to 27 were prepared to evaluate the model's accuracy. Voltammograms were obtained over the range of potential from 1.205 V to 1.719 V under optimized conditions, and subsequent calculations were performed using MATLAB software.

Table 1. Concentration matrix of CAF, TP and TB

Sample	**CAF (10^{-6} mol.L)**	**TB (10^{-6} mol.L^{-1})**	**TP (10^{-6} mol.L^{-1})**
1	6	B2	2
2	8	2	2
3	10	2	2
4	4	4	2
5	6	4	2
6	8	4	2
7	10	4	2
8	12	4	2
9	14	4	2
10	4	6	2
11	6	6	2
12	10	6	2
13	16	6	2
14	4	8	2
15	6	8	2
16	14	8	2
17	8	10	2
18	10	10	2
19	16	10	2
20	4	12	2
21	6	12	2
22	14	12	2
23	16	12	2
24	16	8	2
25	6	10	2
26	8	12	2
27	10	12	2
28	12	12	2
29	16	8	2
30	6	10	2

Calculations using Matlab software were performed with CLS, PLS, PCR to get the CAF, TB concentration in synthesis solutions (Table 2 and Table 3).

Tables 2 and 3 indicate that the PLS method yielded the smallest relative errors, with values below 15%. As a result, the PLS method was chosen for determining CAF and TB in samples using AdSV.

Table 2. Found CAF concentration in mixture solution containing CAF, TP, TB

Sample	[CAF] (μM)	Found content (μM)			Relative error (%)		
		CLS	PLS	PCR	CLS	PLS	PCR
1	8	11.4	7.34	8.19	-43.3	-8.28	-2.48
2	14	15.1	14.4	14.8	-8.00	3.22	-5.88
3	16	13.7	15.5	15.3	14.1	-2.96	4.31
4	6	10.9	6.67	7.79	-83.1	11.1	-29.8
5	8	2.99	7.99	8.06	62.6	-0.13	-0.71
6	10	4.01	9.48	9.90	59.9	-5.24	0.96
7	12	4.01	12.3	11.6	66.6	2.86	3.52

Table 3. Found TB concentration in mixture solution containing CAF, TP, TB

Sample	[TB] (μM)	Found content (μM)			Relative error (%)		
		CLS	PLS	PCR	CLS	PLS	PCR
1	6	4.14	6.77	5.94	31.1	12.8	0.94
2	6	4.27	5.40	5.33	28.8	-9.98	11.2
3	8	9.78	7.60	8.14	-22.3	-5.01	-1.75
4	10	8.01	9.26	7.95	19.9	-7.44	20.5
5	12	12.9	11.5	11.2	-7.83	-3.91	6.74
6	12	14.2	12.6	11.8	-18.0	5.02	1.54
7	12	15.7	12.4	12.7	-30.6	3.57	-5.74

3.2.5. Evaluation of the multivariate regression model validity

By using PLS model, the concentration of CAF, TB, and TP in tea samples were determined based on the concentration matrix of analytes in standard samples. Figure 6 illustrates the relationship between the accumulated percentage of variance explained and the number of PLS components. It is evident that five PLS components accounted for more than 95% of the total variance.

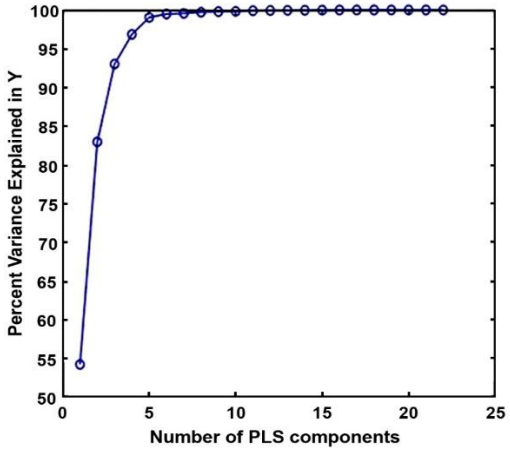

Figure 6. The percent variance explained depended on the number of PLS component

The accuracy of the PLS model, calculated with different numbers of principal components (PCs) was evaluated through the root-mean square error (RMSE) (Table 4).

Table 4. The effect of PC numbers of PLS model to the model's accuracy

Number of PLS	5		6		7	
R^2	0.930	0.967	0.952	0.978	0.943	0.978
RMSE	9.13×10^{-7}	8.15×10^{-7}	6.97×10^{-7}	5.84×10^{-7}	6.77×10^{-7}	5.26×10^{-7}

Table 4 showed that RMSE was lowest when the number of PC was 6. It can be concluded that the developed method can be applied to the simultaneous determination CAF, TB, and TF in tea samples.

3.3. Applications of the proposed method for analysis of tea samples

Tea samples were prepared according to the optimized conditions described above. The analytical results were presented in Table 5. The CAF, TB, and TP content in tea samples was determined by AdSV and compared with the results obtained by HPLC.

Table 5. Concentrations of CAF, TB, TP in tea samples

No	**Samples**	**Content (%)**								
		AdSV			RSD (%) of AdSV			HPLC		
		CAF	TB	TP	CAF	TB	TP	CAF	TB	TP
1	Dai Tu -Thai Nguyen	4.61	0.18	0.30	2.2	3.3	3.6	4.980	0.202	0.339
2	Black Tea - Ha Giang	2.79	0.09	N.D	2.6	3.7	-	2.051	0.095	0.019
3	Xanh Ba green	3.56	0.45	0.24	2.1	3.1	3.8	3.290	0.323	0.224
4	Phu Tho	3.39	0.18	0.31	2.7	3.6	3.1	3.511	0.213	0.336
5	XG –TC– TN	2.73	0.27	0.30	3.5	3.3	2.8	3.028	0.240	0.310
6	Green tea- Cao Bo Ha Giang	3.36	0.28	0.18	2.4	3.1	3.5	3.755	0.330	0.142

Using a paired-t test on the results from the two methods, the p-values obtained were 0.88 for caffeine, 0.78 for theobromine, and 0.68 for theophylline, indicating that the CAF and TP content in tea samples, determined by AdSV, showed no statistically significant difference compared to the HPLC method (at α level of 0.05). The recovery efficiency of the voltammetry was determined by spiking tea samples with standard substances CAF, TP, and TB, ranging

from 94.2% to 102.7%. Based on these results, AdSV coupled with multivariate regression can be applied for the simultaneous determination of CAF, TP, and TB in tea samples.

4. CONCLUSION

A simple voltammetric method using a glassy carbon electrode, combined with the PLS algorithm, was successfully developed for the simultaneous, accurate, selective, and sensitive determination of three methylxanthines caffeine, theobromine, and theophylline in tea samples. This method offers a cost-effective alternative to the widely used HPLC technique, utilizing readily available chemicals and equipment. With recoveries ranging from 94.2% to 102.7% and the smallest relative error of the PLS method below ±11.1%, the proposed approach demonstrates high reliability and precision for the simultaneous determination of caffeine, theobromine, and theophylline in tea samples.

Declarations of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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