

## ***Photoluminescence quantitative analysis of Gallic acid and Caffeine in green tea using multi-way chemometric approaches***

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**ABSTRACT** Green tea is considered as a dietary source of antioxidant nutrients, which acts upon human health. Green tea leaves contain three main components in the form of simple hydroxy benzoic acids such as gallic acid, propyl gallate and xanthic bases (caffeine), have been reported to prevent or delay a number of degenerative diseases and act mainly upon the central nervous system and stimulating wakefulness. Therefore, it is important to establish a simple and reliable analytical method for determination of these compounds in the presence of unexpected interferences in the green tea sample.

In this research, a rapid and sensitive method was used for the direct determination of Gallic acid and Caffeine in green tea that is based on excitation-emission data using chemometric approaches. Multi-way chemometric models can be used to study such data, providing estimates of the spectra and concentration profiles of the underlying chemical analytes. A high percentage of recoveries for the spiked green tea for Gallic acid (i.e. 96.15 %-109.78 %) and Caffeine (i.e. 93.75% -101.57%) indicate the high accuracies of the proposed calibration methods for the assessment of Gallic acid and Caffeine in green tea.

**Keywords:** Green tea, Spectrofluorimetric analysis, Excitation-emission data, Three-way chemometric methods

### **1. INTRODUCTION**

The main phenolic acid of tea is gallic acids [1]. Teas also contain a certain amount of caffeine, which has attracted much scientific and public attention during the past years due to its stimulatory effects. The health effects of Caffeine have been discussed by Trevisanato and Kim [2]. The composition of tea phenolic acids and Caffeine in commercial teas varies with species, season, and horticultural conditions and particularly with a degree of fermentation during the manufacturing process [3, 4]. It is, therefore, important to establish a simple and reliable analytical method for the simultaneous determination of the levels of these compounds in tea samples in developing high quality tea products.

In most of the previous studies, the tea Caffeine has been analyzed separately by TLC [5], GLC [6], HPLC [7, 8–12] or spectrophotometric methods [13]. However, little

research has been reported on the determination of free phenolic compounds, which are also important tea constituents with anti-oxidative properties [7]. Capillary electrophoretic techniques have been developed to separate phenolic and ascorbic acids [14–17].

Fluorescence spectroscopy has become a widely used technique in many fields of chemical, biological and medical sciences [18]. In simple applications, the fluorescence of a fluorophore at a specific pair of excitation and emission wavelengths is used to measure its concentration. However, more often detailed fluorescence scans are used to measure the combined signals from a mixture of known or unknown fluorophores. The mixtures of analyzed fluorophores can vary from simple laboratory mixtures to complex environmental samples [19, 20]. In measuring the fluorescence of mixtures, the fluorescence properties are usually measured by recording measurements spanning both the excitation and emission properties of the mixture. Collating a series of emission scans from a range of excitation wavelengths produces an excitation-emission matrix (EEM) of the sample, representing a detailed map of the fluorescence properties of the mixture. Such EEMs contain a large amount of data, which can in turn hinder the ability of the analyst to utilize all the information collected. Three-way chemometric methods such as parallel factor analysis (PARAFAC) and alternating (APTLD) [21, 22] are powerful techniques for analysis of EEMs, and separate the fluorescence signal of the underlying fluorophores mathematically [21]. PARAFAC and APTLD analyses have been shown to be useful with a wide variety of mixtures of fluorophores [23-26].

This study shows how multivariate data analysis methods can be applied to the study of EEMs for the simultaneous determination of Gallic acid and Caffeine with overlapping spectrum in the presence of interferences not include in calibration set.

## **2. MATERIALS AND METHODS**

### **2.1. MATERIALS**

The entire chemicals used in this investigation were prepared from analytical grade chemicals and distilled water. Sodium dihydrogen phosphate, sodium hydroxide, hydrogen chloride and Gallic acid (purity > 98.0%) were provided from Merck and Caffeine (purity > 98.5%) from Sigma Aldrich.

### **2.2. PREPARATION OF STANDARD AND REAL SAMPLES**

A stock solution of Gallic acid (5000 mgL<sup>-1</sup>) and Caffeine (5000 mgL<sup>-1</sup>) were prepared by dissolving 0.500 g of each compound in buffer solution. The pH of buffer solution was set to 7.5 by dissolving 1.000 g of sodium dihydrogen phosphate in distilled water and was adjusted by addition of 0.1 molL<sup>-1</sup> sodium hydroxide. Six Gallic acid and Caffeine binary

mixtures were prepared by appropriate dilution of stock solution with phosphate buffer. At pH=7.5, the Gallic acid is in acidic and alkaline forms, but the fluorescence intensity of alkaline form is low, therefore the acidic form will be considered in this research.

The real sample was prepared by dissolving 0.100 g of green tea in distilled water and diluted to volume of 100 mL with phosphate buffer solution.

### 2.3. INSTRUMENTATION

A Perkin Elmer LS50B Luminescence spectrofluorometer was used to measure fluorescence landscapes using excitation wavelengths between 240-335 nm with 5 nm intervals with scan speed of 1500 nm/min. The emission wavelength range was 250 to 496 nm with 0.5 nm intervals. Excitation and emission monochromator slit widths were set to 10 nm. The measurement of pH was performed with a Metrohm 691 pH-meter using a combined glass electrode.

### 2.4. PARAFAC MODELING

The data was arranged in I\*J\*K three-way array where the indices refer to the sample, the emission wavelengths and the excitation wavelengths, respectively. The PARAFAC models the data using the following equation:

$$x_{ijk} = \sum_{f=1}^F a_{if} b_{jf} c_{kf} + e_{ijk} \quad i=1, \dots, I; j=1, \dots, J; k=1, \dots, K$$

where  $x_{ijk}$  is the intensity of the  $i$ th sample at the  $j$ th variable (emission mode) and at the  $k$ th variable (excitation mode).  $a_{if}$  is the relative concentration of component  $f$  in the  $i$ th sample,  $b_{jf}$  is the signal intensities at emission wavelength  $j$  and  $c_{kf}$  is the signal intensities at  $k$ th excitation wavelength in each dimension for component  $f$ . The residuals  $e_{ijk}$ , contain the variation not captured by the model [27].

### 2.5. APTLD MODELING

Alternating penalty trilinear decomposition (APTLD) is developed for the decomposition of three-way data arrays. In this algorithm, the constraint functions as penalty terms were used to minimize three new least squares-based constraint objective functions. The value of penalty factors  $p$ ,  $q$  and  $r$  should be chosen before implementation of the APTLD algorithm. The performance of APTLD is regarded to the choice of penalty factors  $p$ ,  $q$  and  $r$ . The proposed algorithm can overcome the slow convergence and being insensitive to increasing number of components by choosing a large number of penalty factors [28-29].

### 2.6. SOFTWARE

The PARAFAC algorithm was performed using the N-way toolbox for decomposition of fluorescence spectra provided by Rasmus Bro in MATLAB environment [27]. The APTLD

algorithm was done using the MVC2 toolbox provided by Olivery [29].

### **3. RESULTS AND DISCUSSIONS**

#### **3.1. SECOND ORDER TRILINEAR DATA GENERATION**

The first step in the three-way methods is providing second order trilinear data. The series of emission scans (250-496) from a range of excitation wavelengths (240-335) were used to generate trilinear fluorescence landscape. The high frequency components of noisy signals can be removed using Hodrick-Prescott smoothing methods regardless of the amplitude.

#### **3.2. RAYLEIGH AND RAMAN SCATTERING REMOVAL**

Figure 1A shows an EEM landscape of the green tea sample. In a fluorescence landscape, the light scattering effects, called Rayleigh (first and second orders are most common) and Raman scattering. When such landscapes are decomposed by three-way analyses, Rayleigh and Raman scatterings may have detrimental effects on the resolved spectra, especially if the peaks from the analytes are close to or on the Rayleigh scattering lines. The contribution of scattering in the EEM spectra can be minimized by elimination of blank water from the data and in some parts of data that are corresponded to scattering, the NAN (not-a-number) values refer to missing data, were used. The use of missing data to replace scattering light in EEMs has been successfully implemented in this study [30]. The obtained result after scattering removal is shown in Figure 2B.

#### **3.3. NUMBER OF ESTIMATED COMPONENTS**

In this work, to estimate the number of variation sources, core consistency diagnostic (CORCONDIA), standard deviation of residuals (SD) were used [31]. In CORCONDIA, when core consistency drops from a high value (approximately more than 90%) to a lower value (approximately fewer than 50%), appropriate number of components have been achieved. In this research for the four-component model, the core consistency diagnostic score was more than 60%. By increasing the number of components, core consistencies close to zero, which indicates the four-component model, provided the greatest spectral resolution for green tea data set. Results of all methods for determining the number of components are shown in Table 1.

### 3.4. IMPLEMENTATION OF THREE-WAY COMPONENT ANALYSIS ON GREEN TEA FLUORESCENCE DATA

For all three-way data arrays analysis, estimation of the emission and excitation profiles is based on DLTD/GRAM decomposition. The optimization procedures of PARAFAC and APTLD are terminated when the following criterion reaches a certain threshold  $\varepsilon$  ( $\varepsilon = 10^{-6}$ ).

$$[(SSR^M - SSR^{M-1}) / SSR^{M-1}] < \varepsilon$$

SSR is the residual sum of squares, and M is the current iteration number.

The loading plots provided by PARAFAC analysis of the EEM data of green tea sample were shown in Figure 2. Results of APTLD with  $p=q=r=10^{20}$ , indicate this algorithm gives satisfactory resolutions for excitation and emission spectral profiles, which are very similar to the loadings obtained from PARAFAC algorithm, but the mean iteration number for APTLD was much less than for PARAFAC.

The linear least squares calibration curves based on the loading matrix corresponding to the sample mode, were provided over the ranges of 1-6 ppm and 3-13 ppm for Gallic acid and caffeine, respectively. Obtained coefficient of determination ( $R^2$ ) from PARAFAC were 0.9335 and 0.9695 for Gallic acid and Caffeine and also 0.9472 and 0.9841 from APTLD for Gallic acid and caffeine, respectively.

After the number of components was estimated, the obtained array by joining the EEMs of the standard and real samples, and those obtained by spiked samples was subjected to decomposition. Comparison of the predicted concentrations and recoveries provided by two algorithms shows a good predictive ability towards the spiked green tea samples, and confirms the potentiality of the multi-way chemometric approaches for the analysis of these complex samples for the assessment of Gallic acid and Caffeine (Tables 2 and 3). Table 4 reports the analytical figures of merit of the two calibration models in a real sample.

## 4. CONCLUSION

In this research, a simple, rapid and selective method was developed for the simultaneous determination of Gallic acid and Caffeine in green tea in the presence of unknown interferences. This method is based on three-way analysis of the excitation-emission fluorescence data and enabled us to handle the direct interfering effect of a green tea matrix. The accuracy of the proposed methods was validated by spiking standard Gallic acid and Caffeine to green tea and recoveries of the spiked value were calculated. These recoveries confirmed the ability of the employed method for the simultaneous determination of Gallic acid and caffeine.

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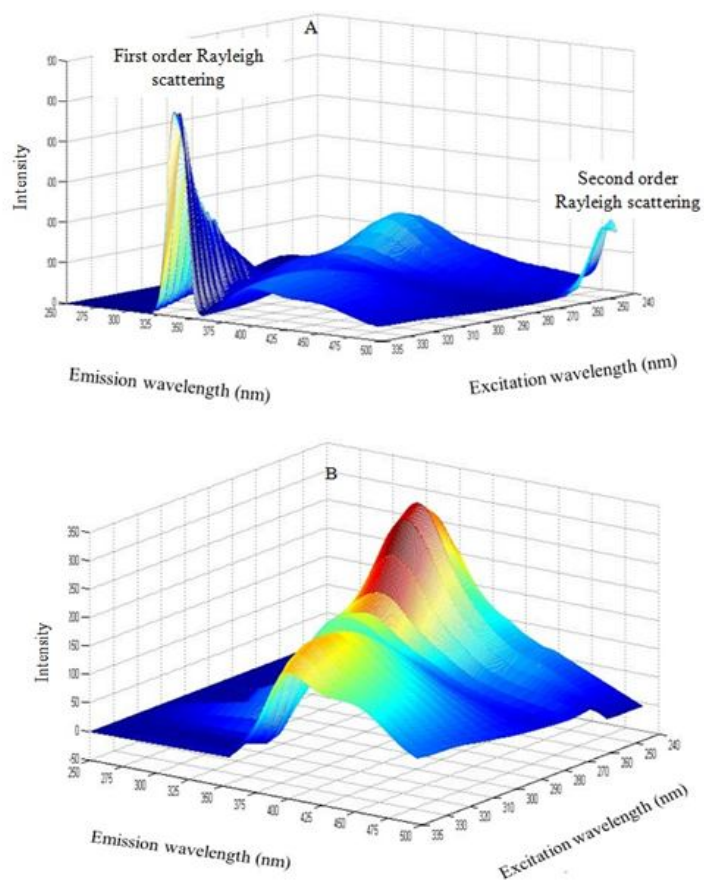
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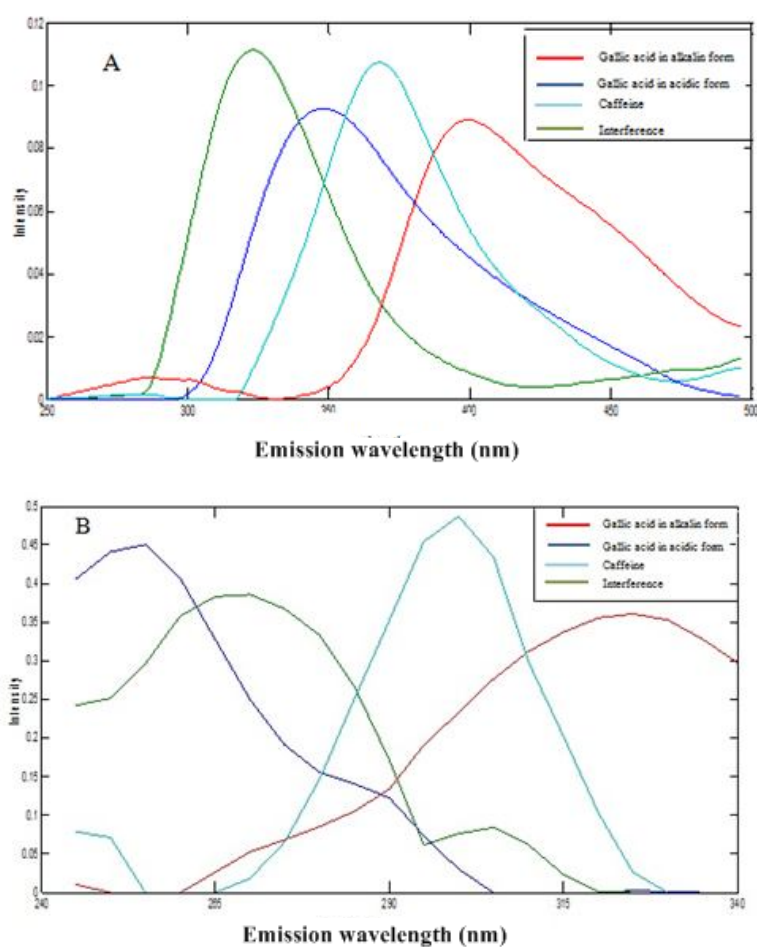
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**Figure 1.** An example of the raw EEM of green tea sample before scattering (A) and after scattering (B) areas have been removed.



**Figure. 2.** Emission (A) and excitation (B) loadings obtained from PARAFAC algorithm.

**Table 1.** Obtained results from different methods in determining the number of components.

Number of components	CORCONDIA(%)	SD of residual
1	100	22
2	99.4	14
3	95.8	6.5
4	63.2	4.8
5	5.2	4.4

**Table 2.** The assessment of Gallic acid in green tea with three way methods.

Method	Added Gallic acid (mgL <sup>-1</sup> )	Found Gallic acid (mgL <sup>-1</sup> )	Recovery (%)	
Real sample	0.00	1.34	-	
	PARAFAC	0.50	2.02	109.78
		1.00	2.25	96.15
Real sample	0.00	1.63	-	
	APTLD	0.50	2.32	108.92
		1.00	2.60	98.86

**Table 3.** The assessment of Caffeine in green tea with three way methods.

Method	Added Caffeine (mgL <sup>-1</sup> )	Found Caffeine (mgL <sup>-1</sup> )	Recovery (%)	
Real sample	0.00	9.51	-	
	PARAFAC	2.00	11.48	99.74
		6.00	14.54	93.75
Real sample	0.00	9.95	-	
	APTLD	2.00	11.43	95.65
		6.00	16.20	101.57

**Table 4.** The analytical figures of merit of the two calibration models in real sample.

		Gallic acid	Caffeine
PARAFAC	Sensitivity	713.79	113.93
	Precision	0.6	0.7
APTLD	Sensitivity	1081.10	206.58
	Precision	0.5	0.5