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Simultaneous Determination of Ibuprofen and Caffeine in Urine Samples by Combining MCR-ALS and Excitation-emission Data

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Second order advantage of excitation-emission fluorescence matrix was applied for the simultaneous determination of ibuprofen and caffeine. The proposed method is based on the measurement of the native fluorescence and recording emission spectra of ibuprofen and caffeine in different excitation wavelengths. The mixture of these compounds was resolved by multivariate curve resolution coupled with alternative least squares (MCR-ALS) on constructed matrix. The EEM spectra were recorded at excitation wavelengths from 250-275 nm; the emission wavelengths ranged from 275-400 nm. For each particular quantitative determination, an augmented matrix was defined. The resolution of each augmented-data matrix gave an estimation of the excitation and emission spectra of the species included in the model. Ibuprofen and caffeine were determined in concentration range from 0.10-8.00 and 0.50-15.00 $\mu\text{g ml}^{-1}$, respectively. The minimal sample pretreatment and relatively low running cost, make this method a good alternative to existing methods for determination of the analytes in urine samples.

Keywords: Ibuprofen, Caffeine, Second order data matrix, Multivariate curve resolution alternative least squares

INTRODUCTION

Ibuprofen (IB); from isobutylphenylpropanoic acid is a nonsteroidal anti-inflammatory drug (NSAID) derivative of propionic acid used for relieving pain, helping with fever and reducing inflammation [1,2,3]. Compared to other NSAIDs it may have fewer side effects such as gastrointestinal bleeding. NSAID works by inhibiting the synthesis of prostaglandins, which are fat-like molecules derived from arachidonic acid involved in mediating inflammation (swelling), pain, and fever. It achieves this effect on prostaglandin synthesis by inhibiting cyclooxygenase, an enzyme that is present in various tissues of the body [4].

Caffeine is a central nervous system stimulant used in combination with other analgesics to improve their efficacy. A combination of ibuprofen and caffeine is used as an effective pain killer [5].

Non-steroidal anti-inflammatory drugs are widely used as analgesics and at higher doses, as anti-inflammatory in the treatment of rheumatic diseases and other musculoskeletal disorders. Besides these actions, NSAIDs act as muddling central nervous system (CNS) depressors. Thus, for preventing this soft CNS depression, central stimulants (*i.e.*, caffeine, CAF) are added sometimes to dosage forms [6,7]. Therefore in this study we investigated simultaneous determination of these two compounds using fluorescence spectroscopy.

In the literature review, due to strong pharmaceutical impact, IB has received particular attention and many methods have been reported for determination of this drug and CF either in their mixture or in mixtures with other drugs. The compounds have been analyzed by different spectrophotometric [8,9], HPLC [10] and spectrofluorimetric methods [11]. The reported spectrofluorimetric method is based on the first derivative spectrofluorimetry using on first order calibration [12].

In first-order calibration methods, both unknown and standard samples should have the same chemical and

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physical characteristics, *i.e.* all detectable species present in the unknown samples, including analytes and interferences, must also be present in the standard samples. This means that standards for calibration must be real samples analyzed beforehand by an independent method. Therefore, the most expensive step in first-order multivariate calibration methods is the preparation and analysis of the large number of standards required for calibration [13].

Fluorescence spectroscopy with two variables (excitation and emission wavelengths) provide a second order advantage for multivariate calibration. Second order methods based on multiple excitation-emission matrix (EEM) fluorescence and multivariate calibration methods can be used to overcome this drawback (constraint of first order method).

Molecular fluorescence is an attractive option, due to its inherent sensitivity and facility in spectral acquisition where reduces the sample pre-treatment steps. In recent years, excitation-emission molecular fluorescence spectroscopy has been used in conjunction with chemometric methods for the analysis of complex samples such as pharmaceutical formulations [14,15].

Fluorimetric data can be arranged in data matrices (*i.e.* second-order data or three ways) for analysis with second-order calibration methods, which may take advantage of features of both excitation and emission spectra of the studied compounds. Second-order instrumentation allows for the analysis in the presence of any component in the sample that is not included in the calibration model. An important advantage of second-order calibration methods is that the analyte can be quantitatively determined using synthetic standard samples containing only the analyte of interest, *i.e.* the interference species in the unknown sample do not need to be present in the standards [16,17].

EEM fluorescence provides data consisting of emission spectra registered at different excitation wavelengths [18]. Therefore, the excitation-emission spectra obtained for each sample can be arranged either in data vector arrays or in data matrices for further analysis that can be used in chemometrics methods [19-21].

In the present study, a second-order multivariate curve resolution procedure based on an alternating least-squares optimization (MCR-ALS) [22-26] is applied to the fluorimetric determination of IB and CF in their mixture.

The MCR-ALS chemometric method enabled IB and CF in samples to be determined on the basis of differences in both excitation and emission spectra of analytes.

EXPERIMENTAL

Apparatus and Materials

The spectra were obtained in a Cary Eclipse Spectrofluorimeter using a 10.00 mm quartz cuvette and the excitation and emission monochromator slit widths were both fixed at 5 nm. All the spectra surfaces of emission/excitation were obtained in the excitation range from 250-275 nm and in the emission range from 275-400.

Ibuprofen and caffeine were kindly donated by the pharmaceutical industries and were used without further purification.

Stock solutions of ibuprofen and caffeine, containing 500 $\mu\text{g ml}^{-1}$ were prepared in 100 ml volumetric flasks, by dissolving 50 mg of each compound in methanol: 0.1 M HCl (3:1) and dilution with distilled water.

Urine sample obtained from healthy volunteer was diluted 100 times in advance. It was assumed that the IB and CF concentration of this urine sample is zero. Urine samples were spiked with appropriate amounts of IB and CF as reported in Table 2.

All calculations were performed in MATLAB 7 (Math Works, Cochituate Place, MA). MCR-ALS codes are available on the internet (Tauler [27]).

Data Sets under Study

The EEM spectra were recorded at excitation wavelengths (λ_{ex}) from 250-275 nm at regular steps of 5 nm; the emission wavelengths (λ_{em}) ranged from 275-400 nm at regular steps of 1 nm. Therefore, for each sample, the excitation-emission raw data matrix measured 126 λ_{em} by 6 λ_{ex} . As an example, Fig. 1 shows a three-dimensional plot of the fluorescence of mixture sample of 5 $\mu\text{g ml}^{-1}$ IB and 10 $\mu\text{g ml}^{-1}$ CF.

Determination of IB and CF in each unknown sample involved the simultaneous analysis of the data matrix of each unknown sample together with the standard matrix. For this purpose, all these matrices were arranged in a new augmented data matrix [25,26]. A particular and important feature of fluorimetry is that each species can be defined by

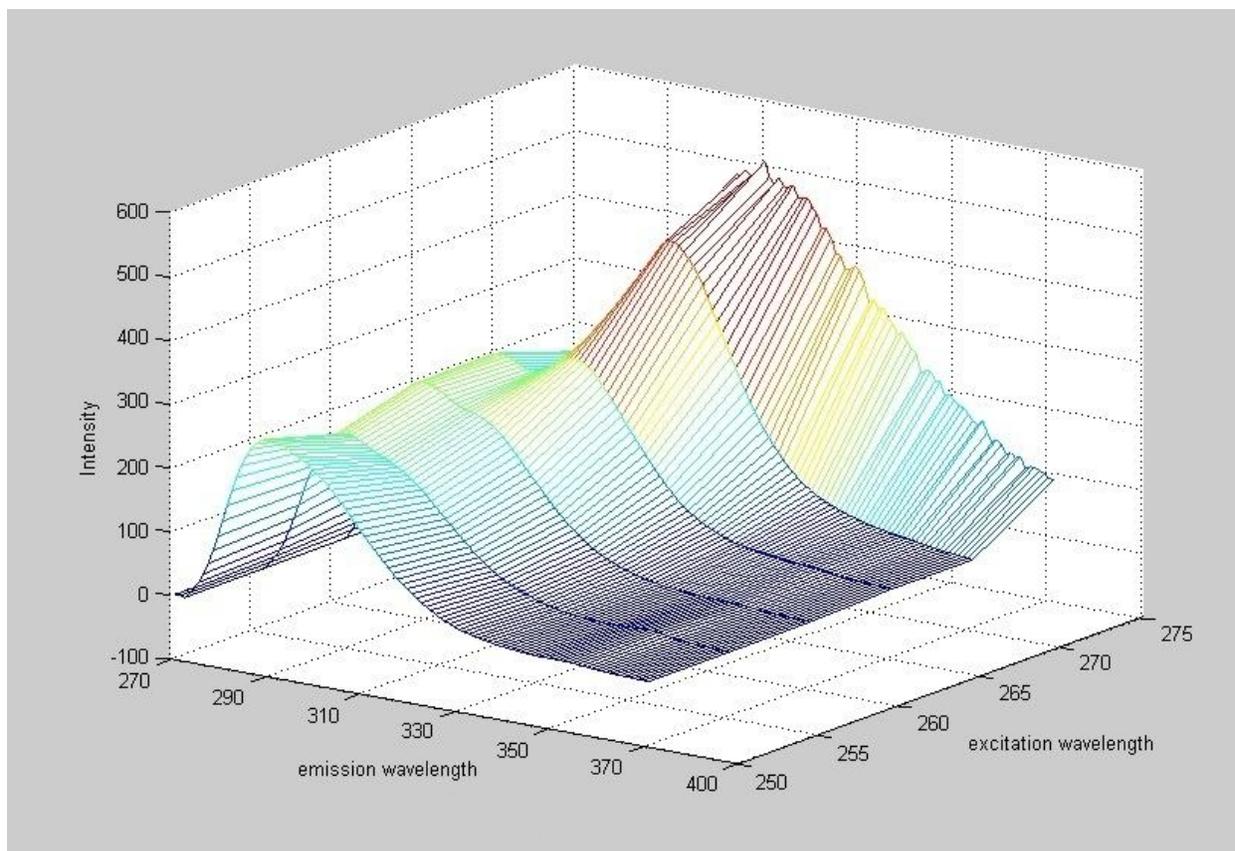


Fig. 1. Three-D plot of the EEM fluorescence of the mixture of 5.00 $\mu\text{g ml}^{-1}$ IB and 10.00 $\mu\text{g ml}^{-1}$ CF.

its characteristic excitation and emission spectra in all data matrices studied simultaneously, so that the data obtained can be assumed to be trilinear [28]. This meant that the second-order data structure could be used to obtain more accurate predictions.

APPLICATION OF THE MCR-ALS METHOD

The determination of IB and CF in samples was performed by a MCR method based on ALS. For each particular quantitative determination, an augmented matrix was defined. The resolution of each augmented-data matrix gave an estimation of the excitation and emission spectra of the species included in the model. The method is based on a linear model (similar to the generalized Beer's law), which

assumes the additivity of the fluorescence of all active compounds. In excitation-wise augmentation the model is as follows:

$$D_{\text{aug}}^{\text{ex}} = Y_{\text{aug}} X^{\text{T}} + E \quad (1)$$

where $D_{\text{aug}}^{\text{ex}}$ is the excitation-wise augmented data matrix, Y_{aug} is the augmented matrix of emission spectra, X^{T} is the transposed matrix of excitation spectra and E is the matrix of residuals.

The aim of the MCR method is to estimate the matrices Y_{aug} and X^{T} from the analysis of D_{aug} only using an ALS optimization. The ALS optimization was started using initial estimates of the excitation spectra of compounds present in the experimental response matrix. [29]. The excitation spectra of both analytes (IB and CF) were

obtained from maximum fluorescence in matrices at the standards of two drugs. These spectra were used as an initial estimate for more constraint and increasing the accuracy of results. These estimates were initially used to calculate the emission spectra as:

$$Y_{\text{aug}} = D_{\text{aug}}^{\text{ex}} * (X^T)^+ \quad (2)$$

Where $D_{\text{aug}}^{\text{ex}}$ is the PCA-reproduced data matrix for the number of components considered, $(X^T)^+$ is the pseudo-inverse of X^T , and Y_{aug} is the augmented matrix of emission spectra updated.

From this new emission spectra matrix Y_{aug} , the excitation spectra were updated using the equation:

$$X^T = (Y_{\text{aug}})^+ D_{\text{aug}}^{\text{ex}} * \quad (3)$$

where $(Y_{\text{aug}})^+$ is the pseudo-inverse of the Y_{aug} matrix.

These two steps were repeated until convergence was achieved. The constraints applied to get physically meaningful solutions during the ALS optimization were: (a) the non-negativity constraint was applied for all excitation and emission spectra (b) the trilinearity constraint was applied for the emission spectrum of each species.

The peak areas of the resolved pure analyte profiles in the standard and in the unknown sample were used as analytical parameters for correlation with the analyte concentrations:

$$C_{\text{un}} = \begin{pmatrix} A_{\text{un}} \\ A_{\text{std}} \end{pmatrix} C_{\text{std}} \quad (4)$$

where C_{un} and C_{std} are the concentrations of the analyte in the unknown and standard samples, respectively; A_{un} and A_{std} are the areas below the excitation or emission spectra profiles in the unknown and in the standard samples, respectively.

The calibration line of each analyte was obtained from the plotting of the analyte responses in different calibration samples vs. their corresponding analyte concentration. Then the analyte concentration in an unknown sample was predicted by using the calibration graph and the peak area of the analyte in the unknown sample.

A prediction sample set was synthesized to evaluate the prediction ability of the proposed method. The overall prediction error was calculated as the statistical parameter for the validation of the applied method defined as follows:

$$\text{Error}(\%) = \frac{\sqrt{\sum_{i=1}^{\text{sample}} (C_{\text{true}} - C_{\text{calc.}})^2}}{\sqrt{\sum_{i=1}^{\text{sample}} (C_{\text{calc.}})^2}} \times 100 \quad (5)$$

where C_{true} is the true concentration of analyte in the sample, and $C_{\text{calc.}}$ is the concentration calculated by the proposed method [13].

RESULTS AND DISCUSSION

Ibuprofen and Caffeine have native fluorescence with maximum emission at 290-335 nm, respectively (Fig. 2). Different solvents and media were tried such as water, methanol, 0.1 M HCl, 0.1 M NaOH, acetate buffer (pH 4.5), phosphate buffer (pH 7.5, 9.5), and the maximum fluorescence intensity was obtained in a mixture of methanol and water. Previous reports of spectrofluorimetric determination of CF were based on solid phase determination because of its low fluorescence intensity [12], but in the proposed method increased sensitivity in minimum amount of methanol allowed to determine this drug in the solution (20:80 methanol:water).

Analysis of Synthetic Samples

A set of sample solutions with different drug concentrations were prepared in the ranges 0.1-8.00 and 0.5-15.00 $\mu\text{g ml}^{-1}$ for IB and CF, respectively. Then, the EEMs were augmented in the excitation mode and analyzed by MCR-ALS. The number of chemical species present in each EEM was first estimated by singular value decomposition, since it was assumed that chemical components were associated with the largest singular values. The number of species finally chosen was checked to provide a chemically reliable resolution of the system. The chemical rank of the standard matrix of IB and CF was found to be 2.

Figure 3 shows the results of the MCR-ALS resolution of the excitation-wise augmented data matrix $[U^T; S^T]$ composed of the unknown sample (U), calibration samples reported in Table 1.

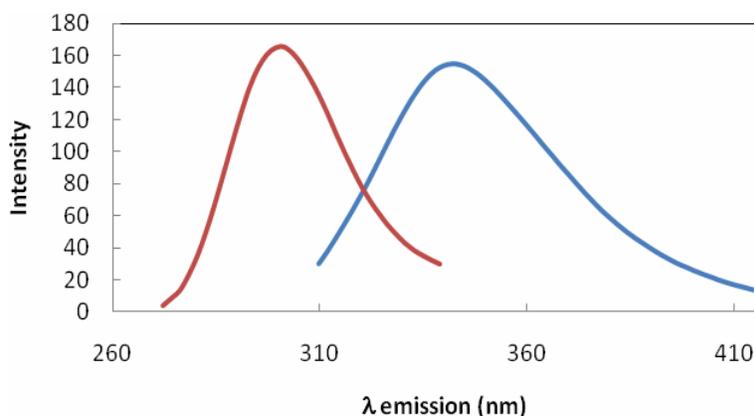


Fig. 2. Emission spectra for 3.00 µg ml⁻¹ IB and for 5.00 µg ml⁻¹ CF, λ_{ex} = 260 nm.

Table 1. Analytical Characteristics for the Analysis of IB and CF in Methanol

Analyte	Calibration equation	R ^{2b}	Linear range	LOD
			(µg ml ⁻¹)	(µg ml ⁻¹)
IB	F = 34.29C ^a + 16.45	0.991	0.10-8.00	0.052
CF	F = 10.04C + 14.36	0.987	0.50-15.00	0.180

^aµg ml⁻¹. ^bSquared calibration coefficient.

Table 2. The Application of the Proposed Method for Simultaneous Determination of IB and CF after Addition to the Urine Samples

Sample	Spiked (µg ml ⁻¹)		Found (µg ml ⁻¹)		Recovery (%)		RSD (%) (n = 3)	
	IB	CF	IB	CF	IB	CF	IB	CF
	Urine	0.00	0.00	ND ^a	ND	-	-	-
	1.00	0.50	0.91 ± 0.02 ^b	0.52 ± 0.03	91.00	104.00	2.80	2.43
	5.00	5.00	5.12 ± 1.12	4.91 ± 0.85	102.40	98.20	3.67	2.98
	8.00	10.00	8.21 ± 2.05	9.76 ± 2.18	102.63	97.60	3.93	3.56

^aND: not detected. ^bStandard deviation for three determination.

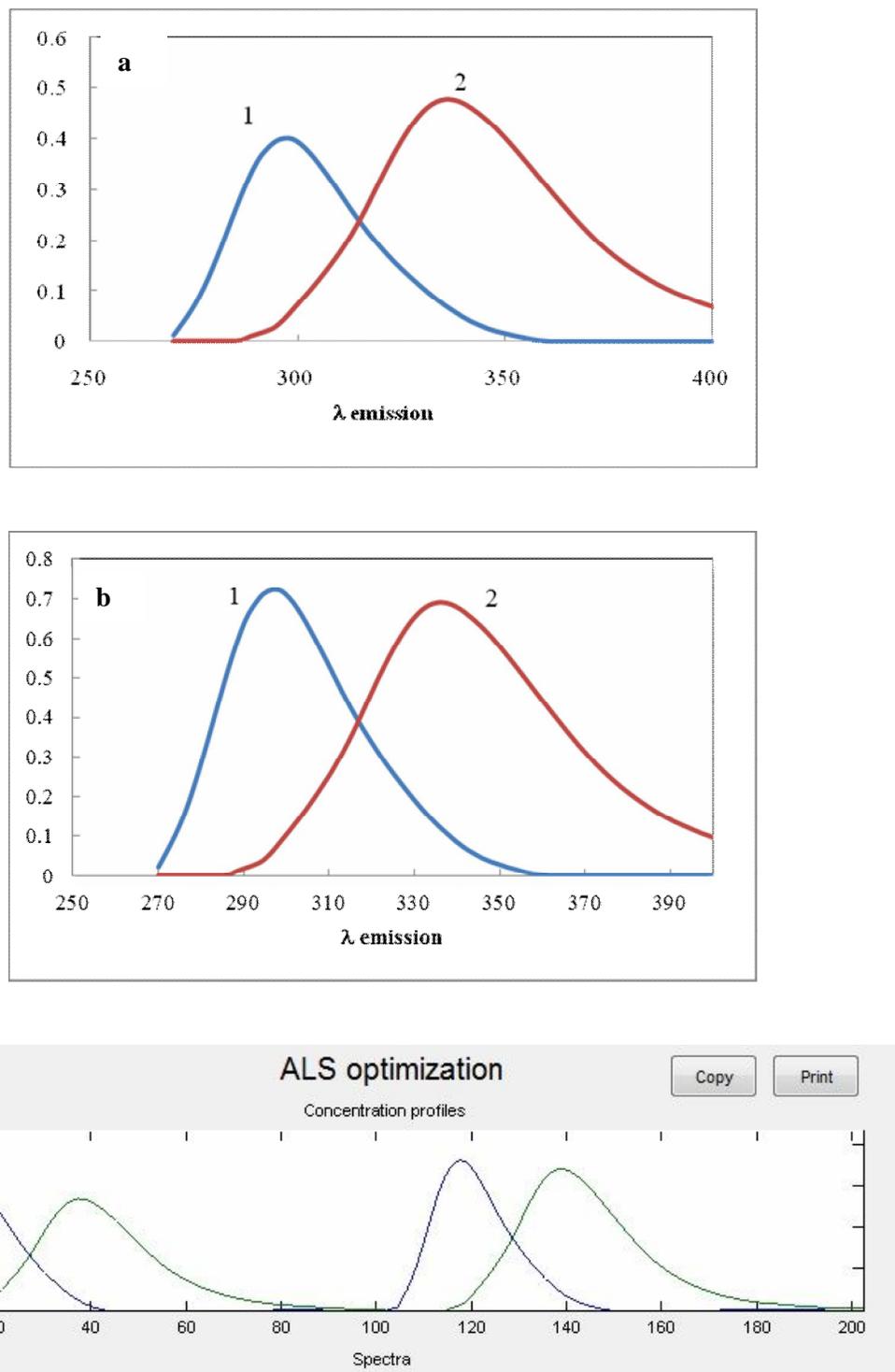


Fig. 3. Emission spectra recovered in the resolution of the excitation-wise augmented matrix $[U^T; S^T]$ using the ALS-MCR method. Species assignment: 1. IB; 2. CF Emission spectra for the unknown sample (matrix U) (a); emission spectra for the standard sample (matrix S) (b); MCR output (c).

The shape of the emission spectra recovered for each species in the different matrices was exactly the same, as a consequence of the trilinear structure of the data and of the application of trilinearity constraints during the ALS optimization [23]. The method allowed the emission spectra of IB and CF to be estimated, as well as the estimation of their excitation spectra.

The calibration line of each analyte was plotted whose equation is given in Table 1, where F is peak emission area of each analyte in the calibration samples and C is the concentration of analyte ($\mu\text{g ml}^{-1}$). The limit of detection (LOD) was defined as the compound concentration that produced a signal-to-noise ratio greater than three. Based on this criterion LOD values were $0.05 \mu\text{g ml}^{-1}$ and $0.18 \mu\text{g ml}^{-1}$ for IB and CF, respectively.

Note that, in this case, the quantitative information was contained in the emission spectra and that IB and CF in the unknown sample were determined by using the peak area of the emission spectrum of the analyte for the unknown sample and its calibration line.

Based on Eq. (5) the overall prediction error of the test set was 4.20%.

Application of the Method in Spiked Urine

Urine without the spiked analytes showed emission spectra with maximum wavelength in 390 nm which overlapped slightly with the spectra of analytes. As we select the data matrix up to 400 nm the fluorescence of urine does not show large effect for determination of two components.

Two components with different concentrations (three additions) were added simultaneously to the urine sample and after augmentation of the matrices and resolving them with MCR-ALS method, the concentrations of them were determined and the recovery of each analyte was calculated. The results are given in Table 2. The calculated recoveries were between 90.80-107.60%.

CONCLUSIONS

In the proposed method, combination of EEM and MCR-ALS could be applied with a great success for the simultaneous determination of IB and CF with the second order advantage. It was proven that the developed methods

are highly efficient in resolving the two components in a way that is comparable to separation techniques such as HPLC. This is the first report on simultaneous spectrofluorimetric determination of IB with another compound using second order advantage of fluorescence. The potential advantages of this determination, such as rapidity and low cost, can be even more highlighted by considering the possibility of automating the proposed method.

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