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# PLS AND PARAFAC APPLIED TO DETERMINATION OF NOSCAPINE IN BIOLOGICAL FLUIDS BY EXCITATION – EMISSION MATRIX FLUORESCENCE

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A method for the direct determination of noscapine, an opium alkaloid, in biological fluids such as urine and human plasma has been developed using excitation-emission matrix fluorescence measurements and chemometrics techniques based on partial least squares (PLS) and parallel factor (PARAFAC) analysis. A two-factor PARAFAC trilinear model without restrictions was used in the data analysis. The excitation wavelength range was from 285 to 315 nm and the emission was recorded from 325 to 500 nm. The calibration graph was constructed using sixteen standard solutions in the concentration range from 0.50 to 21.00 μg/ml for noscapine. The capabilities of the method for the analysis were evaluated by determining noscapine in synthetic and real samples with satisfactory results. In addition, a PLS model was built at one excitation wavelength and used to determine noscapine in a series of synthetic and real samples. The accuracy of the proposed methods, evaluated through the root mean square error of prediction (RMSEP) for noscapine by PARAFAC and PLS models was 0.2375 and 0.4901, respectively. The best model was obtained using PARAFAC analysis. This result shows that molecular fluorescence spectroscopy can be used for the development of robust analytical methods for the direct determination of noscapine on a complex background such as urine and human plasma.

## *INTRODUCTION*

Noscapine is the second most abundant alkaloid in opium, which is present in concentrations within 2 – 8% [1] and is used in antitussive drug compositions. Unlike morphine and codeine, noscapine has no analgesic activity or abuse potential. Its major pharmaceutical action is the antitussive effect, which has been reported to be equivalent to that of codeine [2]. Recent studies indicated that noscapine may cause apoptosis in many cell types and has potent antitumor activity against solid murine lymphoid tumors and human breast and bladder tumors implanted in nude mice [3]. The British Pharmacopoeia (BP) procedures [4, 5] for the quantitative determination of noscapine in pharmaceutical preparations are based on the optical absorption method, which lacks specificity, and the acid – base titrimetric method in a non-aqueous medium with potentiometric and end-point detection. Neither method is suitable for determining low levels of the alkaloid [6]. Other previously published analytical assays include high-performance liquid chromatography (HPLC) [7 – 9], liquid chromatography [10, 11] and spectrophotometry [6, 12]. For practical applications, it is necessary to develop a simple and more sensitive method of noscapine determination.

To the best of our knowledge, there were no reports on noscapine determination by fluorescence techniques. However, the high sensitivity of fluorescence spectroscopy made it a useful analytical tool for monitoring trace compounds of biochemical interest. The quantification of an analyte in presence of unknown or uncalibrated interferences can be achieved by means of three-way (and beyond) models, according to which each sample is represented by a data matrix or a second-order signal [13 – 15]. Fluorescence has the character of generated second-order signals with the corresponding the excitation – emission matrix (EEM), which consist of emission spectra recorded at various excitation wavelengths. The information given by fluorescence in combination with chemometrics techniques, such as parallel factor (PARAFAC) [16] and partial least squares (PLS) [17] analysis, allows one to study complex samples under fairly general conditions without having to calibrate or know the interferences present beforehand.

We determined noscapine in biological fluids such as urine and human plasma using PARAFAC deconvolution of three-dimensional (3D) excitation – emission data and PLS calibration of the two-dimensional (2D) data. The aim of this paper is to show that excitation-emission matrix fluorescence spectroscopy and parallel factor analysis (PARAFAC) can be used for distinguishing between synthetic and real samples (urine and human plasma). The selectivity of the proposed method was evaluated from recovered spectra. Next, a univariate regression was performed for noscapine by relating the loading of the PARAFAC decomposition with the known concentrations of the noscapine in the calibration set of samples. Finally, the accuracy of the proposed method was estimated by predicting a new set of samples, which were not used at the calibration step.

## *THEORY*

PARAFAC analysis [18 – 20] is a multiway method originating from psychometrics. It is gaining more and more interest in chemometrics and associated areas for many reasons: increased awareness of the method and its possibilities, increased complexity of the data dealt with in science and industry, and increased computational power. Essentially, PARAFAC analysis represents one of several decomposition methods for *N*-way data, offering a genera-

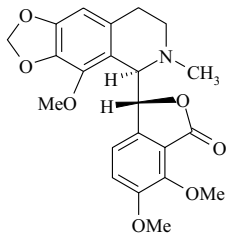

**Fig. 1.** Chemical structure of noscapine.

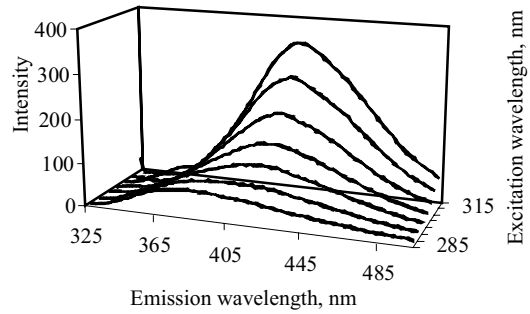


**Fig. 2.** Typical EEMF spectra of noscapine (pH 7.0).

lization of the principal component analysis (PCA) to higher orders. It can be considered a constrained version of the more general method Tucker-3, with and identity core matrix. It is less flexible, uses fewer degrees of freedom, and provides a unique solution independent of rotation. This last feature is a great advantage in the modeling of spectroscopic data. The true underlying spectra (or whatever constitutes the variables) will be found if the data is in fact trilinear, the right number of components is used, and the signal-to-noise ratio is appropriate.

**Three-way decomposition of fluorescence data**. Fluorescence three-way data can be decomposed using PARAFAC analysis because each analyte in the sample can ideally be described by one PARAFAC component. This means that each fluorophore contribution to the emission is independent of the contribution of the remaining fluorophores and identical for different samples (only varying in proportions). Hence, the PARAFAC model for a three-way array ($x_{ijk}$) can be denoted as

$$x_{ijk} = \sum_{f=1}^{F} a_{\mathrm{if}}\, b_{jf}\, c_{kf} + e_{ijk}. \qquad (1)$$

In the case of excitation – emission matrix fluorimetry (EEMF), the $k$ th slice of the trilinear cube $\underline{X}$ is an $I \times J$ matrix of excitation and emission profiles of the fluorescent components for the $k$ th sample. Thus, $a_{\mathrm{if}}$, $b_{\mathrm{jf}}$, and $c_{\mathrm{kf}}$ are the typical elements of the loading matrices $A$, $B$, and $C$ (emission wavelength, excitation wavelength, and relative analyte concentrations in the samples, respectively) for a given number of components $F$. Using an alternative least squares (ALS) procedure, the trilinear model is found to minimize the sum of squares of residues $e_{ijk}$. Using the matrix notation and the Khatri – Rao product [18], the PARAFAC array can be written as

$$\underset{(IJ\times K)}{\underset{\sim}{X_k}} = (\underset{(J\times F)}{\underset{\sim}{B}} |\otimes| \underset{(I\times F)}{\underset{\sim}{A}})\, \underset{(K\times F)}{\underset{\sim}{C}}^T = \underset{(IJ\times F)}{\underset{\sim}{Z}}\, C^T \qquad (2)$$

An important characteristic of the PARAFAC model is the uniqueness of its solution. This means that additional constraints, such as orthogonality or external information to solve rotational freedom are not needed to identify the model [18]. This property is an extension of the second-order advantage and so trilinear data (here fluorescence data) cab be calibrated when there are unknown interferences in the samples. In dilute solutions or suspensions, fluorescence intensity is linearly proportional to the solute concentration, and fluorescent excitation-emission measurements follow a trilinear model, such as the PARAFAC.

**PARAFAC calibration and prediction**. Decomposition of the three-way data by PARAFAC gives rise to three loading matrices, one of which (matrix $C$) corresponds to the sample mode. The $C$-loadings represent the relative concentrations of noscapine in solutions. In the calibration step, these loadings are regressed against the real concentrations of noscapine in solutions used to obtain a linear calibration graph [21]. In the prediction step, this regression line can then be used to predict the concentration of noscapine in future test samples [21]. The theory and application of PARAFAC analysis in spectrophotometry have been discussed, for example, in [22 – 28].

**Partial least squares** offer a method [29] for building regression models based upon the latent variable decomposition relating two blocks, matrices $X$ and $Y$, which contain the independent ($x$) and dependent ($y$) variables, respectively. These matrices can be simultaneously decomposed into a sum of $f$ latent variables as follows:

$$X = TP^T + E = \sum_{i}^{n} t_f p'_f + E, \qquad (3)$$

$$X = UQ^T + F = \sum_{i}^{n} u_f q'_f + F, \qquad (4)$$

where $T$ and $U$ are the score matrices for $X$ and $Y$, respectively; $P$ and $Q$ are the loadings matrices for $X$ and $Y$, respectively; and $E$ and $F$ are the corresponding residual matrices. The two matrices are correlated with the scores $T$ and $U$ for each latent variable as

$$u_f = b_f t_f, \qquad (5)$$

where $b_f$ is the regression coefficient for the $f$ th latent variable. Matrix $Y$ can be calculated from $u_f$ as Eq. (5), and concentrations of the new samples can be estimated from the new scores $T^*$, which are substituted in Eq. (5) to yield

$$Y = TBQ^T + F, \qquad (6)$$

$$Y_{\mathrm{new}} = T^* BQT. \qquad (7)$$

In this procedure, it is necessary to find the best number of latent variables, which normally is performed using cross-validation based on determination of the minimum

prediction error. Application of PLS in spectrometry has been discussed in [30 – 32]. In addition, several determinations based on the application of these methods to spectroscopy data have been reported in [33 – 37].

**Statistical parameters**. For evaluating the predicting ability of a multivariate calibration model, the root mean square error of prediction (RMSEP) and relative standard error of prediction (RSEP) can be used [27], which are defined as

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n}(y_{pred} - y_{obs})^2}{n}}, \quad (8)$$

$$RSEP(\%) = 100 \times \sqrt{\frac{\sum_{i=1}^{n}(y_{pred} - y_{obs})^2}{\sum_{i=1}^{n}(y_{obs})2}}, \quad (9)$$

where $y_{pred}$ is the predicted concentration in the sample, $y_{obs}$ is the observed value for the same sample, and $n$ is the number of samples in the validation set.

## *EXPERIMENTAL PART*

**Reagents**. Noscapine, phosphoric acid, boric acid, acetic acid, hydrochloric acid, and sodium hydroxide were purchased from Merck. All reagents were of analytical and reagent grade. A standard (stock) solution of noscapine with a concentration of 1000 μg/ml was prepared by dissolving an appropriate weighed amount of the compound in water. This solution was stored in the dark at 4°C and was found to be stable for at least four weeks, albeit changing in the spectral profile. All solutions were prepared using deionized water. Universal buffer solution was prepared according to [38].

**Instrumentation and software**. All fluorescent measurements were carried out on a Carry Eclipse fluorescence spectrophotometer (Varian), equipped with a 150-W xenon lamp and connected to a microcomputer running under Windows-XP. Data acquisition was performed by the use of Carry software. In all cases, 1.00-cm quartz cells were used. The excitation wavelength range was varied from 285 to 315 nm at a 5-nm step and the emission was recorded in a range from 325 to 500-nm every 2 nm, thus making a total of 7 × 87 = 609 data points per sample matrix. The excitation and emission slit widths were both 4 nm, and a scan rate of 500 nm/min was used. A Metrohm 692 pH-meter furnished with a combined glass-saturated calomel electrode was calibrated with at least two buffer solutions at pH 3.00 and 9.00.

The data were treated in an AMD 2000 XP (256 Mb RAM) microcomputer using MATLAB (Version 6.5) software (MathWorks). The *N*-way toolbox for MATLAB (Version 2.1) [available at http://www.models.kvl.dk/source] was employed for PARAFAC calculations, while PLS calculus was carried out using the PLS-Toolbox (Version 2.0) (Eigenvector Technologies).

**Solution preparation**. Known amounts of standard solutions were placed in a 10-ml volumetric flask and completed to the mark with deionized water and universal buffer at pH 8.0. The final concentration of these solutions varied between 0.50 to 21.00 μg/ml for noscapine.

**Analysis of biological samples**. Urine spiked with noscapine was obtained by the following procedure: an aliquot of pure noscapine was added to 10-ml urine sample. A 1 ml aliquot of the resulting urine solution was mixed with 5 ml (0.2 M) sodium carbonate buffer and 10 ml butyl chloride. The mixture was rotated for 20 min and centrifuged at 2000 rpm for 10 min. The butyl chloride layer was separated and evaporated to dryness. The resultant residue was dissolved in universal buffer (pH 7.0) in a 10 ml volumetric flask, and diluted to the mark [27].

Plasma spiked with noscapine was obtained by diluting aliquots of the standard stock noscapine solution with the human plasma. A 1 ml aliquot of this spiked solution was diluted to 5 ml with ethanol in 10 ml centrifuge tube. The precipitated protein was separated by centrifugation for 10 min at 2500 rpm. The clear supernatant layer was filtrated via Whatman filter to obtain protein-free spiked human plasma [27], and then it was added into 10 ml volumetric flask and diluted to the mark by universal buffer (pH 7.0).

## *RESULTS AND DISCUSSION*

**Excitation-emission matrix of noscapine**. A three-dimensional plot of the EEM of noscapine in the range $\lambda_{ex}$ = 285 – 315 nm, $\lambda_{em}$ = 325 – 500 nm is presented in Fig. 2. As can be seen, there is the main band at the excitation/emission wavelengths about 315 nm/425 nm. Since the Rayleigh scattering is not related to trilinear EEMF, these areas were deleted. The very intense peak at $\lambda_{em}$ = 325 – 500 nm is attributed to noscapine. The measu-

Table 1

**Concentrations (μg/ml) of the Calibration and Prediction Sets of Noscapine Solutions for PARAFAC and PLS Models**

| Calibration | Concentration | Calibration | Concentration | Prediction | Concentration |
|---|---|---|---|---|---|
| C1 | 0.50 | C11 | 9.00 | P1 | 0.70 |
| C2 | 0.75 | C12 | 10.00 | P2 | 1.10 |
| C3 | 1.00 | C13 | 11.00 | P3 | 2.30 |
| C4 | 1.50 | C14 | 12.00 | P4 | 5.50 |
| C5 | 2.00 | C15 | 13.00 | P5 | 8.60 |
| C6 | 2.50 | C16 | 14.00 | P6 | 10.50 |
| C7 | 4.00 | C17 | 15.50 | P7 | 15.00 |
| C8 | 5.00 | C18 | 17.00 | P8 | 16.00 |
| C9 | 7.00 | C19 | 19.50 | P9 | 18.00 |
| C10 | 8.00 | C20 | 21.00 | P10 | 20.00 |

Table 2

**Percentage Fit and CORCONDIA Values for PARAFAC Models with Various Numbers of Components**

| Number of factors | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Fit, % | 93.45 | 99.88 | 99.89 | 99.90 | 99.91 |
| CORCONDIA,% | 100.00 | 93.04 | 3.24 | 0.31 | 0.18 |

rements were performed in the entire accessible wavelength range. A universal buffer solution of pH 7.0 was selected. In order to select the optimum pH value at which the intensity reaches maximum, the influence of the pH of medium on the emission spectra at various excitation wavelengths were studied over a pH range from 2.0 to 11.0.

*PARAFAC Analysis*

The main advantage of three-way multivariate calibration is that it allows the information about concentration of an individual component to be extracted in the presence of any number of uncalibrated constituents. Therefore, this technique is highly useful for solving analytical problems involving a complex matrix (Fig. 2). The data were arranged in a three-way array ($30 \times 87 \times 7$) for 30 solutions with variable noscapine concentrations (Table 1) in the rows, 87 emission wavelengths in the columns, and 7 excitation wavelengths in the slices. No preprocessing (centering or auto scaling) was applied to the data. When using PARAFAC analysis, an initial definition of the number of factors used to build the model is necessary. This choice is of fundamental importance, since all conclusions about the deconvolution and quantitation results will be related with this number of factors. In PARAFAC analysis, it is possible to use several constraints such as non-negativity, unimodality, or orthogonality. In this work, an unconstrained model was preferred because more realistic results can be obtained. Unconstrained PARAFAC models of the noscapine data at various different excitation wavelengths were developed using one to five components and the percentage fit was used as the initial approach to select the number of factors. The percentage fit value indicates how well the model can reproduce the experimental data and it is defined as [16]

$$f_i(\%) = 100 \times \left( 1 - \frac{\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}(X_{ijk} - \hat{X}_{ijk})^2}{\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} X_{ijk}^2} \right), \quad (10)$$

Table 3

**Statistical Characteristics of Linear Relationships between the Proportion Loadings Calculated Using PARAFAC Analysis and the True Concentrations of Noscapine Solutions**

| Characteristic | First *C*-loading (first calibration) | Second *C*-loading (second calibration) |
|---|---|---|
| Number of data points | 20 | 20 |
| Intercept | 0.1314 | 0.0844 |
| Standard deviation of intercept | 0.0102 | 0.0189 |
| Slope | 0.6945 | 0.30455 |
| Standard deviation of slope | 0.0013 | 0.0039 |
| Correlation coefficient | 0.9992 | 0.9971 |
| Standard deviation of regression | 0.0346 | 0.0424 |

where $X_{ijk}$ is the *ijk* th experimental element and $\hat{X}_{ijk}$ is the *ijk* th element predicted by the model. The results are presented in Table 2. As can be seen, this parameter is not critical for selecting the number of factors, since percentage fits higher than 99% were obtained using from 2 to 5 factors. This parameter is important to determine if there are factors lacking in the model. For this reason, other more conclusive tools such as CORCONDIA were used in this study.

**Core consistency diagnostics (CORCONDIA)**. The entire data set ($30 \times 87 \times 7$) was utilized for the core consistency evaluation using one to five factors calculated according to Eq. (11). The CORCONDIA parameter is defined as [16]:

$$\text{CORCONDIA} = 100 \times \left( 1 - \frac{\sum_{d=1}^{F}\sum_{e=1}^{F}\sum_{f=1}^{F}(gdef - t_{def})^2}{\sum_{d=1}^{F}\sum_{e=1}^{F}\sum_{f=1}^{F} t_{def}^2} \right), \quad (11)$$

where $g_{\text{def}}$ is the core element calculated using the PARAFAC model (defined by dimensions $(d \times e \times f)$; $t_{\text{def}}$ is the element of a binary array with zeros in all elements and unities in the super-diagonal; and $F$ is the number of factors in the model. In the ideal PARAFAC model, $g_{\text{def}}$ is equal $t_{\text{def}}$ and, in this case, CORCONDIA value will be 100%. The appropriate number of factors is accessed by a model with the maximum number of factors and valid CORCONDIA test. An analysis of the core consistency confirms that two factors are necessary, because the use of a greater number of factors leads to a great decrease in the core consistency. Three factors give a CORCONDIA value of 100% (a perfect trilinear model), whereas for three or more factors this value decreases below 4%. These results are also shown in Table 2.

**Deconvolution and calibration**. According to CORCONDIA calculations, two factors for unconstrained PARAFAC model provide the best results for the deconvolution of data. The decomposition of the three-way data by PARAFAC gives rise to three loading matrices, one of which ($C$) corresponds to the sample mode. The $C$-loading gives the relative concentrations of noscapine in the solutions. In the calibration step, these loadings are regressed against the real concentrations of noscapine so as to obtain a linear calibration.

By plotting these $C$-loadings versus real concentrations of noscapine, two calibration curves were obtained for which the calibration results are shown in Table 3. The average recoveries for the PARAFAC procedure were 98.7% for the first calibration curve and 97.7% for the second curve. According to the results obtained using the two calibration curves, the first calibration is more suitable. Data on the linear regression, standard deviation of results, line equations, and correlation coefficients are summarized in Table 3. In the prediction step, this regression line can be used to predict the concentration of noscapine in future test samples. The results obtained by applying PARAFAC analysis to eight synthetic samples are listed in

Table 4, which also shows the recovery for prediction series of noscapine solutions, the RMSEP, and the RSEP values. As can be seen, the results of prediction for noscapine are very good.

*PLS analysis*

**Calibration and validation**. The multivariate calibration is a powerful tool for determinations, because it extracts more information from the available data and allows building more robust models. Therefore, it was decided to perform a multivariate calibration using PLS models built for a single excitation wavelength (315 nm) and the emissions recorded from 325 to 500 nm, and compare this calibration with PARAFAC models. According to the experimental plan (Table 1), 20 solutions were used to construct the models (calibration set) and another 10 solutions to validate them (validation set).

**Selecting the optimum number of factors**. The optimum number of factors (latent variables) to be included in the calibration model was determined by computing the prediction error sum of squares (PRESS) for cross-validated models using a large number of factors (half the number of total standard plus one), which was defined as follows:

$$PRESS = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2, \qquad (12)$$

where $y_i$ is the reference concentration for the $i$ th sample and $\hat{y}_i$ is the estimated concentration. The cross-validation method employed was to eliminate only one sample at a time and then perform PLS calibration of the remaining standard spectra. Using this calibration, the concentration of the sample left out was predicted. This process had been repeated until each standard was left out once.

One reasonable choice for the optimum number of factors would be that corresponding to the minimum PRESS. Since there are a finite number of samples in the training set, the minimum PRESS value in many cases causes overfitting for unknown samples, which were not included in the model. A solution to this problem has been suggested by Haaland et al. [39, 40], according to which the PRESS values for all previous factors are compared to the PRESS value at the minimum. The *F*-statistics test can be used to determine the significance of PRESS values that exceed the minimum.

The maximum number of factors used to calculate the optimum PRESS was selected as 11, and the optimum number of factors obtained by the application of PLS model is three (PRESS = 0.1156). In all instances, the number of factors for the first PRESS values whose *F*-ratio probability drops below 0.75 was selected as the optimum.

**Determination of noscapine in synthetic solutions**. The predicting ability of both PLS model at one excitation wavelength (315 nm) and all emission wavelengths were determined using 10 synthetic solutions (their compositions are listed in Table 4). The results obtained by applying PLS at a single excitation wavelength to 10 synthetic samples are also given in Table 4, together with the RMSEP and RSEP values. According to these results, the PARAFAC model is better than the PLS model.

**Determination of noscapine in biological fluids**. In order to show the analytical applicability of the proposed methods, first calibration curves obtained from PARAFAC and PLS at a single excitation wavelength were applied to the determination of noscapine in real samples (urine and human blood plasma). The results (Table 5) showed that satisfactory recovery for noscapine could be obtained using the PARAFAC procedure. The data obtained by this method reveal the ability of this method to determine noscapine in urine and human plasma samples without considerable error.

In conclusion, this work demonstrated that the EEMF method in combination with multiway analysis is a powerful tool for the complex analysis of noscapine in urine and plasma, since, in most cases, this compound produces fluorescence. Deconvolution by means of the PARAFAC analysis resulted in two factors, one due the noscapine and the other due to two urine or plasma constituents. It was demonstrated that the conjugation of several procedures such as percentage fit and CORCONDIA analysis leads to a more realistic estimation of the number of factors in PARAFAC models. The encouraging results of this exploratory analysis suggest that the study could be extended to

Table 4

**Added and Found Noscapine Concentrations (μg/ml) in the Prediction Set Determined Using PARAFAC and PLS Methods**

| Added noscapine | PARAFAC model | | | | PLS model | |
|---|---|---|---|---|---|---|
| | Found* | Recovery*, % | Found** | Recovery**, % | Found | Recovery, % |
| 0.70 | 0.67 | 95.7 | 0.59 | 84.9 | 0.54 | 77.1 |
| 1.10 | 1.06 | 93.4 | 1.01 | 91.8 | 0.98 | 89.1 |
| 2.30 | 2.35 | 102.2 | 2.41 | 104.9 | 2.11 | 91.7 |
| 5.50 | 5.56 | 101.1 | 5.59 | 101.6 | 5.16 | 93.8 |
| 8.60 | 8.45 | 98.3 | 8.36 | 97.2 | 8.16 | 94.9 |
| 10.50 | 10.23 | 97.4 | 10.21 | 97.2 | 10.22 | 97.3 |
| 15.00 | 14.89 | 99.3 | 14.53 | 96.9 | 14.33 | 95.5 |
| 16.00 | 16.12 | 100.8 | 16.33 | 102.1 | 16.76 | 104.8 |
| 18.00 | 18.33 | 101.8 | 18.59 | 103.3 | 18.69 | 103.8 |
| 20.00 | 19.43 | 97.2 | 19.39 | 96.9 | 20.66 | 103.3 |
| RMSEP | 0.2375 | | 0.3510 | | 0.4901 | |
| RSEP | 1.9889 | | 2.9389 | | 4.1034 | |

**Note**.* First and ** second calibrations according to Table 3.

Table 5

**Determination of Noscapine Concentrations (μg/ml) in Urine and Human Blood Plasma Samples by EEMF Using PARAFAC and PLS Models**

| Sample | Added | PARAFAC (First calibration) | | | PLS | | |
|---|---|---|---|---|---|---|---|
| | | Amount found | RSD* | Recovery, % | Amount found | RSD* | Recovery, % |
| Urine 1 | 1.00 | 0.94 | 3.34 | 94.0 | 0.81 | 4.19 | 81.0 |
| Urine 2 | 5.00 | 4.87 | 3.05 | 97.4 | 4.23 | 4.13 | 84.6 |
| Plasma 1 | 1.50 | 1.39 | 3.11 | 92.6 | 1.11 | 3.89 | 74.0 |
| Plasma 2 | 7.50 | 7.38 | 2.89 | 98.4 | 6.94 | 3.48 | 92.5 |
| Plasma 3 | 10.00 | 9.47 | 2.12 | 94.7 | 9.06 | 3.66 | 90.6 |

the development and application of threeway methods to EEMF and other second-order data.

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## ФЛУОРОМЕТРИЧЕСКОЕ ОПРЕДЕЛЕНИЕ НОСКАПИНА В БИОЛОГИЧЕСКИХ ЖИДКОСТЯХ С ИСПОЛЬЗОВАНИЕМ МЕТОДОВ ЧАСТИЧНЫХ НАИМЕНЬШИХ КВАДРАТОВ И ПАРАЛЛЕЛЬНОГО ФАКТОРНОГО АНАЛИЗА

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Описана методика флуорометрического определения опиумного алкалоида носкапина в биологических жидкостях (плазма крови, моча) с использованием хемометрического подхода на основе методов частичных наименьших квадратов (partial least squares, PLS) и параллельного факторного анализа (parallel factor analysis, PARAFAC). Флуорометрический анализ предложено проводить с возбуждением в области длин волн 285 – 315 нм и детектированием в области 325 – 500 нм. Калибровочные кривые построены для 16 растворов носкапина в интервале концентраций 0.50 – 21.00 мкг/мл с использованием PLS (при одной длине волны) или PARAFAC (с трехмерной матрицей данных для разных длин волн возбуждения и эмиссии). Возможности предложенной методики проверены путем анализа модельных и реальных растворов с известными добавками носкапина. Наилучшие результаты были получены с использованием параллельного факторного анализа: стандартные ошибки определения носкапина составили 0.2375 (PARAFAC) и 0.4901 (PLS). Молекулярная флуоресцентная спектроскопия и хемометрический подход к обработке данных обеспечивают надежное определение носкапина в сложных биологических объектах, таких как плазма крови и моча.