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# Simultaneous Determination of codeine and noscapine by flow-injection chemiluminescence method using *N*-PLS regression

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ABSTRACT

A flow injection chemiluminescent (FI-CL) method has been developed for the simultaneous determination of codeine and noscapine using *N*-PLS regression. The method is based on the fact that kinetic characteristics of codeine and noscapine are different in the $Ru(phen)_3^{2+}$–Ce(IV) CL system. In flow injection mode, codeine gives broad peak with the highest CL intensity at 4.4 s, whereas the maximum CL intensity of the noscapine appears at about 2.6 s. Moreover, the effect of increasing $H_2SO_4$ concentration was different on the CL intensity of the compounds. An experimental design, central composite design (CCD), was used to realize the optimized variables such as Ru(II) and Ce(IV) concentrations for the both compounds. At the optimized condition, a three-way data structure (samples, $H_2SO_4$ concentration, time) was constructed and followed by *N*-PLS regression. The number of factors for the *N*-PLS regression was selected based on the minimum values for the root mean squared error of cross validation (RMSECV). The proposed method is applied to the simultaneous quantification of codeine and noscapine in the pharmaceutical preparations.



## 1. Introduction

Opiates are the most powerful known pain relievers; they are derivatives of opium and can produce euphoria. In addition they are used as analgesics. Codeine and noscapine belong to the poppy alkaloid group of opiates. Codeine is a derivative of morphine and it is qualified as a drug abuse, which is used as a local anesthetic in many pharmaceutical preparations. Noscapine (narcotine) is the second most abundant alkaloid in opium, present in concentrations of 2–8% [1]. Unlike codeine, noscapine has no analgesic activity or abuse potential. These two drugs have been used for decades as antitussive in cough syrups and their antitussive activity is alike [2].

Several analytical techniques have been applied for the simultaneous determination of codeine and noscapine such as LC–MS [3–5], TLC [6], CE [7,8], HPLC [9,10] or individual of them [11]. However, there is not any report for the simultaneous chemiluminescence (CL) determination of these alkaloids without using a separation technique up to now.

CL is an attractive method with characteristics such as a low detection limit, fast response, and wide linear dynamic range. In addition, the instruments are relatively simple [12]. However, the method suffers from the lack of selectivity [13]. Several techniques have been used to increase the specificity of CL analysis such as the use of masking agents [14], chromatography [15], and wavelength discrimination [16].

The CL methods have also been used for the determination of codeine [17] or noscapine [1].

Generally, simultaneous determination of compounds by CL methods, without using a separation technique, could be conducted by time resolved CL or chemometric-assisted methods. Ruiz et al. [18] developed a manual time-resolved CL method for the simultaneous determination of the binary mixtures of citrate and pyruvate using batch mode. The method was based on the different rates of the CL reaction of these acids in $Ru(bpy)_3^{2+}$–Ce(IV) CL system. The same reagents and method have also been used for the determination of oxalate-tartrate [19] and pyruvate-tartrate [20]. Pulgarin et al. [21] also described a stopped flow technique for the simultaneous determination of morphine and naloxone in synthetic samples. In these methods, influence of sample matrix in selected times should be investigated for each component to ensure that the slop of the calibration curve of one analyte not affected by another [21].

Chemometrics methods (generally partial least squares (PLS) algorithm) have also been used in CL methods for the simultaneous determination of analytes in mixture. For example, PLS has been used for the simultaneous determination of cobalt and copper [22], protocatechuic and caffeic acids [23], cobalt and chromium [24–26], ascorbic acid and L-cysteine [27] and morphine along with naloxone [28].

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Multi-way PLS (*N*-PLS) algorithm, which has been developed by Bro [29], maintains the three or higher dimensional structures of the data, with the capability of extracting more information of a kinetic system than the conventional PLS. The advantages of the three way data respect to the two way data have been investigated by Bro [30]. One of the main limitations for applying *N*-PLS in CL methods is attributed to the lack of the wavelength separation techniques in the common CL instruments. Therefore, unlike to spectrophotometric methods, wavelength couldn't be applied in most CL methods as a variable or discrimination factor. Consequently in CL methods, spectral information has not employed for using in multi-way methods such as *N*-PLS and constructing a there or higher dimensional data is relatively difficult.

In this work, a $H_2SO_4$ concentration mode was added to the time and sample modes to obtain the three way data. A simple flow injection (FI) method, instead of batch or stopped flow, was used for the simultaneous CL determination of these two alkaloids in pharmaceutical formulations using *N*-PLS regression. In addition predictive ability of the *N*-PLS model has been compared with conventional PLS model.

## 2. Theory

### 2.1. N-PLS

The theoretical aspects of *N*-PLS method have been described in several books and reviews [29–31]. In summary, multi-way regression method, *N*-PLS extends the traditional PLS algorithm to higher orders, using the multi-dimensional structure of the data for model building and prediction [29]. In the case of three-way data, the model is given by the following equation:

$$x_{ijk} = \sum_{f=1}^{F} t_{tf} \boldsymbol{w}^{J}_{jf} \boldsymbol{w}^{K}_{kf} + e_{ijk} \quad (1)$$

Where $x_{ijk}$ is the CL intensity measured for sample $i$ at pH $j$ and time $k$, $F$ is the number of factors, $t_{tf}$ is an element of the score matrix $\boldsymbol{T}$, $w^{J}_{jf}$ and $w^{K}_{kf}$ are elements of two $\boldsymbol{w}$ loading matrices and $e_{ijk}$ is a residue not fitted by the model. The model finds the scores yielding maximum covariance with analyte concentrations as the dependent variable, in a three dimensional sense. One of the advantages of using *N*-PLS over bi-dimensional regression is a stabilization of the decomposition involved in Eq. (1), which potentially gives increased interpretability and better predictions.

## 3. Experimental

### 3.1. Apparatus

The schematic diagram of the FI system is shown in Fig. 1. The CL signal was measured with a CL analyzer with PMT (Model $R_{212}$, Hammamatso, Japan) and a low pass filter whose output was connected to a data processing system. A 12-channels peristaltic pump (Desaga, Model PLG, USA) with three silicon rubber tubes (1.0 mm i.d.) was used. PTFE mixing joints and PTFE tubing (1.0 mm i.d.) were used for the connections. The flow cell was U shape with 0.6 mL inner volume and 0.5 cm distance from the PMT. Sample solutions were injected using a six positions rotary valve.

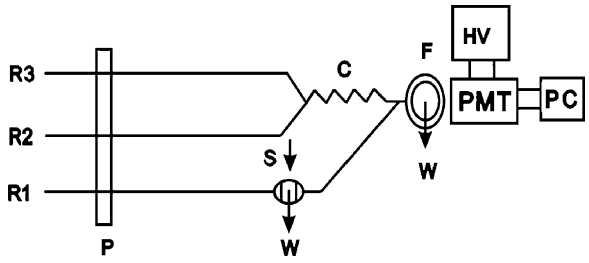


**Fig. 1.** Schematic diagram of FI system, $R_1$: $H_2O$, $R_2$: acidic Ce(IV), $R_3$: Ru(II), P: peristaltic pump, S: injection valve, C: reaction coil, F: flow cell, W: waste, HV: high voltage power supply, PMT: photomultiplier tube, PC: computer.

### 3.2. Reagents

All the solutions were prepared using reagent grade chemicals and doubly distilled water. Noscapine and codeine standard solutions ($1.0 \times 10^{-3}$ mol $L^{-1}$) were daily prepared by dissolving 0.0415 g of noscapine base (Temad Co., Iran) and 0.0410 g of codeine phosphate (Temad Co., Iran) in 100.0 mL volumetric flasks. Ru(II) solution ($1.0 \times 10^{-2}$ mol $L^{-1}$) was prepared by dissolving 0.3640 g of dichlorotris (1, 10-phen) ruthenium(II) hydrate (Sigma–Aldrich, Steinheim, Germany) in 50.0 mL water. Ce(IV) solutions ($2.3 \times 10^{-3}$ mol $L^{-1}$) were prepared by dissolving 0.1270 g of ceric ammonium nitrate (Riedel-de Haën, Germany) in proper volumes of 1.0 mol $L^{-1}$ $H_2SO_4$ and diluting to the mark with distilled water in 100.0 mL volumetric flasks. In this way, $H_2SO_4$ concentrations of 0.03, 0.05, 0.08 and 0.12 mol $L^{-1}$ were prepared.

### 3.3. Sample preparation

One mL of each syrup drug was directly transferred into a 100 mL volumetric flask and diluted to the mark. Each time 5.0 mL of this solution and a proper volume of the standard solution (including codeine and/or noscapine) were transferred into a 25 mL volumetric flask and diluted to the mark.

### 3.4. Calibration set

Based on our primary experiments, the CL intensity versus concentration was linear in the range $5.0 \times 10^{-6}$ to $5.0 \times 10^{-5}$ mol $L^{-1}$ for both compounds. In this range, a 4-level full factorial experimental design was used for preparation of standard solutions. Concentration of codeine and noscapine in the binary mixtures, used for *N*-PLS regression are given in Table 1.

**Table 1**
Composition of standard solutions used for the multi-way regression.

| Sample No. | Codeine (mol $L^{-1}$) | Noscapine (mol $L^{-1}$) | Sample No. | Codeine (mol $L^{-1}$) | Noscapine (mol $L^{-1}$) |
|---|---|---|---|---|---|
| 1 | $5.0 \times 10^{-6}$ | $5.0 \times 10^{-6}$ | 9 | $5.0 \times 10^{-6}$ | $3.5 \times 10^{-5}$ |
| 2 | $2.0 \times 10^{-5}$ | $5.0 \times 10^{-6}$ | 10 | $2.0 \times 10^{-5}$ | $3.5 \times 10^{-5}$ |
| 3 | $3.5 \times 10^{-5}$ | $5.0 \times 10^{-6}$ | 11 | $3.5 \times 10^{-5}$ | $3.5 \times 10^{-5}$ |
| 4 | $5.0 \times 10^{-5}$ | $5.0 \times 10^{-6}$ | 12 | $5.0 \times 10^{-5}$ | $3.5 \times 10^{-5}$ |
| 5 | $5.0 \times 10^{-6}$ | $2.0 \times 10^{-5}$ | 13 | $5.0 \times 10^{-6}$ | $5.0 \times 10^{-5}$ |
| 6 | $2.0 \times 10^{-5}$ | $2.0 \times 10^{-5}$ | 14 | $2.0 \times 10^{-5}$ | $5.0 \times 10^{-5}$ |
| 7 | $3.5 \times 10^{-5}$ | $2.0 \times 10^{-5}$ | 15 | $3.5 \times 10^{-5}$ | $5.0 \times 10^{-5}$ |
| 8 | $5.0 \times 10^{-5}$ | $2.0 \times 10^{-5}$ | 16 | $5.0 \times 10^{-5}$ | $5.0 \times 10^{-5}$ |

### 3.5. Experimental procedure

The solutions containing carrier $H_2O$ ($R_1$), Ce(IV) solution in sulfuric acid ($R_2$) and Ru(II) solution ($R_3$) were pumped at 2.8 mL $min^{-1}$ (for each channel) via a peristaltic pump. At joint S, an aliquot (200 μL) of standard solution consisting the both compounds was injected into carrier stream by a sample injection valve. $R_2$ and $R_3$ solutions were mixed through 10 cm reaction coil (silicon tube, 1.0 mm i.d.) and then mixed with the sample solution exactly in front of PMT to produce peak-like CL emission that is monitored by a computer.

The three way data were obtained by recording CL intensity of sixteen bi-component mixture solutions at 4 different sulfuric acid concentrations including 0.03, 0.05, 0.08 and 0.12 mol $L^{-1}$. The CL intensity for each sample made up from 1000 point of time with time intervals of 13 ms. After recording the CL responses, the initial time ($t = 0$) for all of them adjusted to 10 points before rising the CL peak and 900 points after rising the CL peak. In this way, a three way data with dimensions of [16 × 4 × 1000] was obtained.

### 3.6. Software

All computations were performed using Matlab (The Math. Works Inc., Natick, MA, USA) and the *N*-PLS analysis was carried out by using the *N*-way Toolbox for Matlab, freely accessible via Internet [32].

## 4. Results and discussion

### 4.1. Kinetic profile of CL reaction of $Ru(phen)_3^{2+}$–acidic Ce(IV)-noscapine and codeine

The methodology of the method is based on the fact that the reduction rates of noscapine and codeine in the CL reaction of $Ru(phen)_3^{2+}$ and acidic solution of Ce(IV) are different. Generally, in batch mode, the maximum CL signals of codeine and noscapine appear at 3 and 0.5 s after the injection of Ce(IV) solution, respectively. The CL signal of codeine is a broad peak, whereas the CL signal of noscapine is a sharp and intense peak. Kinetic profiles of codeine, noscapine and their mixture in batch mode are shown in Fig. 2.

The batch mode provides a good efficiency of time-resolved CL [18–20], but in this mode, reproducibility intensively is affected by the efficiency of mixing. Therefore, the precision of the method is reduced. As in our primary investigations, RSDs larger than 15% were obtained for noscapine at concentrations lower than $1.0 \times 10^{-5}$ mol $L^{-1}$.

Flow injection analysis (FIA) provides a better reproducibility. Typical CL profiles of codeine, noscapine and their mixture in FI mode are shown in Fig. 3. Like the batch mode, the shapes of the CL profiles in FI mode are different, and in comparison with codeine, a more rapid rising and decaying signal observes for noscapine. However, the peaks are overlapped more than the batch mode.

Based on the previous works [33–35], the CL mechanism could be suggested as follows:

$$Ru(phen)_3^{2+} + Ce(IV) \rightarrow Ce(III) + Ru(phen)_3^{3+}$$

$$Ru(phen)_3^{3+} + (NoscapineorCodeine) \rightarrow [Ru(phen)_3^{2+}]* + (Noscapine^{+}orCodeine^{+})$$

$$[Ru(phen)_3^{2+}]* \rightarrow Ru(phen)_3^{2+} + h\nu$$

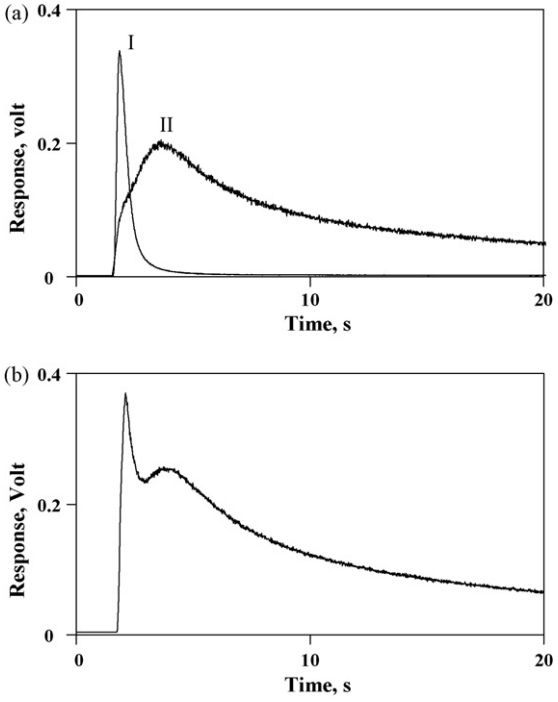


**Fig. 2.** Kinetic profiles in batch mode, a) I: $1.0 \times 10^{-5}$ mol $L^{-1}$ of noscapine and II: $1.0 \times 10^{-5}$ mol $L^{-1}$ of $1.0 \times 10^{-5}$ mol $L^{-1}$ of their mixture; concentrations of $H_2SO_4$, Ru(II) and Ce(IV) were 0.012 mol $L^{-1}$, $8.0 \times 10^{-4}$ mol $L^{-1}$ and $8.0 \times 10^{-4}$ mol $L^{-1}$, respectively.

### 4.2. Influence of chemical variables

Concentration of $H_2SO_4$ made a different effect on the CL intensity of codeine or noscapine. At low concentrations of $H_2SO_4$ (~0.03 mol $L^{-1}$), CL intensities of both codeine and noscapine were weak but CL of noscapine was more intense than that of codeine. As $H_2SO_4$ concentration was increased, the CL intensities of both compounds varied, but with different rates. As can be seen in Fig. 4, CL intensity of codeine at $H_2SO_4$ concentrations lower than 0.08 mol $L^{-1}$ is very dependent to $H_2SO_4$ concentration and whereas in the higher concentration, no dependence to $H_2SO_4$ concentration. Inversely, CL intensity of noscapine alters rapidly at both lower and higher concentrations of 0.08 mol $L^{-1}$ $H_2SO_4$. $H_2SO_4$ concentration wasn't optimized because it was used for constructing the three way data as mentioned in section 3.5.

To find the compromised optimum concentrations for Ce(IV) and Ru(II) that be proper for codeine, noscapine and their mixture, we used a two factor-five level ($\alpha = 2$) central composite design (CCD). The design is shown in Fig. 5 and coded values along with

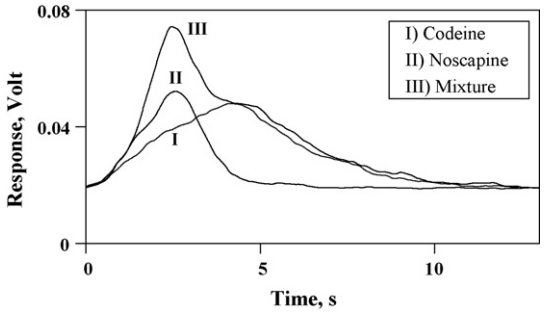


**Fig. 3.** Kinetic profiles in FI mode; Concentrations of $H_2SO_4$, Ru(II) and Ce(IV) were 0.05 mol $L^{-1}$, $3.5 \times 10^{-3}$ mol $L^{-1}$ and $2.3 \times 10^{-3}$ mol $L^{-1}$, respectively. Codeine and noscapine concentrations were $1.0 \times 10^{-5}$ mol $L^{-1}$.

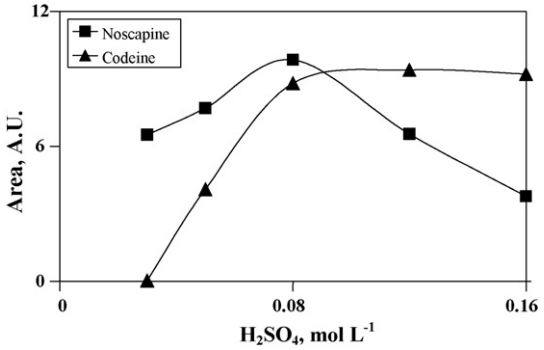


**Fig. 4.** Influence of $H_2SO_4$ concentration on the codeine and noscapine CL responses; Area under the CL profiles obtained for each experiment.

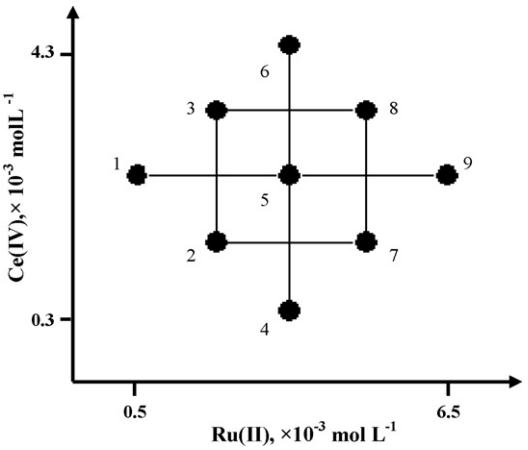


**Fig. 5.** CCD experimental design for selecting concentrations of Ce(IV) and Ru(II) for simultaneous determination of codeine and noscapine.

their respective actual levels for each point in the CCD experimental design are given in Table 2. After constructing the design, CL response of codeine, noscapine and their mixture recorded separately at each one of 9 conditions of the design. Therefore totally 27 experiments were performed. As can be seen in Fig. 6, concentrations of Ce(IV) and Ru(II) corresponding to point number 4 in the design ($Ce(IV) = 0.3 \times 10^{-3}$ mol $L^{-1}$ and $Ru(II) = 3.5 \times 10^{-3}$ mol $L^{-1}$) have produced maximum CL response for the noscapine and the mixture but in this condition sensitivity wasn't good for codeine. Therefore, conditions corresponding to point number 5 ($Ce(IV) = 2.3 \times 10^{-3}$ mol $L^{-1}$ and $Ru(II) = 3.5 \times 10^{-3}$ mol $L^{-1}$) were selected for simultaneous determination of codeine and noscapine because in those concentrations of Ce(IV) and Ru(II), the CL

**Table 2**
Coded and actual values used by 2 factors-5 levels CCD for the optimization of Ru(II) and Ce(IV).

| Point No. | Coded values | | Actual values ($\times 10^{-3}$ mol $L^{-1}$) | |
|---|---|---|---|---|
| | Ru(II) | Ce(IV) | Ru(II) | Ce(IV) |
| 1 | −2 | 0 | 0.5 | 2.3 |
| 2 | −1 | −1 | 2.0 | 1.3 |
| 3 | −1 | +1 | 2.0 | 3.3 |
| 4 | 0 | −2 | 3.5 | 0.3 |
| 5 | 0 | 0 | 3.5 | 2.3 |
| 6 | 0 | +2 | 3.5 | 4.3 |
| 7 | +1 | −1 | 5.0 | 1.3 |
| 8 | +1 | +1 | 5.0 | 3.3 |
| 9 | +2 | 0 | 6.5 | 2.3 |

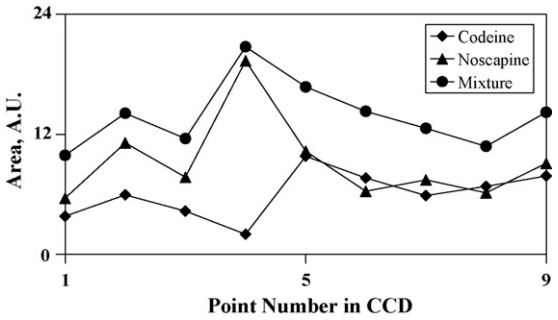


**Fig. 6.** Influence of Ce(IV) and Ru(II) in determination of codeine and noscapine. Area under the CL profiles, obtained for each set of conditions of CCD, was used as response. Concentration of $H_2SO_4$ was 0.08 mol $L^{-1}$.

responses were appropriate for all of solutions consisting codeine, noscapine and their mixture.

### 4.3. N-PLS regression

Since, the CL kinetic profiles of codeine and noscapine are overlapped with each other a first or second order calibration is required to predict the concentration of the each compound in the mixture. It was realized that the effect of $H_2SO_4$ concentration in a definite range (0.03–0.12 mol $L^{-1}$) had different influences on the CL intensity of the compounds. Therefore, it was thought that concentration of $H_2SO_4$ has potential to be selected as a new variable for constructing a three way data instead of working with two way data. In this regard, a three-way data structures, [sample, $H_2SO_4$ concentration, time], was constructed. The next step was selecting a suitable multi-way regression model. There are different multi-way methods such as PARAFAC and *N*-PLS. However, as the *N*-PLS regression model uses the dependent and independent variables for constructing the models, therefore its predictive ability is better than the PARAFAC models. In addition, trilinearity is not essential for the *N*-PLS model. Therefore, the *N*-PLS regression model was constructed between the concentrations of noscapine and codeine as the dependent variables and the three way data.

### 4.4. Number of factors

The performance of *N*-PLS model was evaluated by calculating the root mean squared errors of cross validation (RMSECV) for each analyte, which is defined as follows [36]:

$$\mathrm{RMSECV} = \sqrt{\frac{\sum_1^N (y_i - \hat{y}_i)^2}{N}} \quad (2)$$

In that, $y_i$ is the reference concentration for the *i*th sample and $\hat{y}_i$ represents the predicted concentration. In RMSECV method, one sample was eliminated at a time and then *N*-PLS model constructed with remaining standard samples. By using this calibration, the concentration of the sample, left out, was predicted. This value

**Table 3**
Explained variance for X and Y blocks and RMSECV for different number of factors.

| | Number of Factors | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| X block (%)[a] | 88.63 | 95.23 | 96.18 | 98.02 | 98.28 |
| Y block (%)[b] | 84.56 | 98.85 | 99.25 | 99.38 | 99.54 |
| RMSECV (codeine, $\times 10^{-6}$) | 7.5 | 4.1 | 1.1 | 2.8 | 2.8 |
| RMSECV (noscapine, $\times 10^{-6}$) | 3.2 | 0.6 | 1.7 | 4.5 | 4.7 |

[a] X block: The three way data.
[b] Y block: Concentrations of noscapine and codeine.

**Table 4**
Effect of foreign substances in determination of $2.0 \times 10^{-5}$ mol $L^{-1}$ of noscapine or $1.0 \times 10^{-5}$ mol $L^{-1}$ of codeine.

| Substance | Molar ratio[a] | |
|---|---|---|
| | Noscapine | Codeine |
| Sucrose, glucose, saccharine, serine, valine, $K^+$, phenylalanine, $NO_3^-$, lactose, fructose, threonine, leucine, $CN^-$, $CO_3^{2-}$, $Br^-$ | 100 | 100 |
| Proline, aspartic acid, $Na^+$, $Zn^{2+}$ | | 50 |
| EDTA, $Cl^-$, $Cu^{2+}$, $Cr^{3+}$, $Cd^{2+}$ | 50 | 10 |
| $Ca^{2+}$, $Fe^{2+}$, $Mg^{2+}$ | 10 | |
| Histidine, tryptophane | 5 | 1 |
| $I^-$, morphine | 0.5 | 0.1 |

[a] Molar ratio of substance to noscapine or codeine.

was calculated for different number of the factors in the model. The results are listed in Table 3. The optimum number of the factors was selected based on the minimum value for the RMSECV. It can be noticed that the RMSECV values are minimum for three and two factors for codeine and noscapine, respectively. The number of the factors for relating codeine concentration to the data is larger than the number of the analytes. This could be related to the non-linearity in the data, which could be compensated by enhancing the number of the factors in the model. Beyond the respective number of factors for codeine and noscapine, the model was overfitted.

### 4.5. Influence of foreign compounds

To evaluate the selectivity of the proposed method, the influence of the foreign species on the determination of noscapine and codeine was separately investigated. The tolerance of each substance was taken as the largest amount yielding an error of less than 5% in the analytical signal of $1.0 \times 10^{-5}$ mol $L^{-1}$ of codeine or $2.0 \times 10^{-5}$ mol $L^{-1}$ of noscapine. The results are listed in Table 4.

In addition, binary mixtures of both analytes along with common excipients such as lactose, sucrose, saccharine and sodium chloride were studied using *N*-PLS model. The procedure consisted of preparing different solutions with each one of these excipients in different amounts up to concentration of $5.0 \times 10^{-4}$ mol $L^{-1}$ and containing codeine and noscapine at $1.0 \times 10^{-5}$ mol $L^{-1}$ and $2.0 \times 10^{-5}$ mol $L^{-1}$, respectively. The *N*-PLS models were used to predict the concentrations of codeine and noscapine in the presence of the excipients. The results are presented in Table 5. It was realized that these excipients in concentrations lower than $5.0 \times 10^{-4}$ mol $L^{-1}$ were not able to alter the response values and therefore, they were not interfering compounds in analysis of codeine and noscapine.

**Table 5**
Predicted concentration of codeine ($1.0 \times 10^{-5}$ mol $L^{-1}$) and noscapine ($2.0 \times 10^{-5}$ mol $L^{-1}$) in presence of each excipient ($5.0 \times 10^{-4}$ mol $L^{-1}$) using *N*-PLS.

| Excipients | Recovery (%) | |
|---|---|---|
| | Codeine | Noscapine |
| Lactose | 102.4 | 111.1 |
| Sucrose | 101.0 | 100.6 |
| Saccharin | 108.2 | 96.7 |
| Sodium Chloride | 93.3 | 104.5 |

### 4.6. Application

In order to investigate the accuracy of the method, three cough syrup samples were analyzed to determine codeine and noscapine contents. In this regard, samples were prepared as described in the sample preparation section, and the predicted concentrations were obtained by the *N*-PLS model. The results are given in Table 6. It can be noticed that the recoveries are in the range of 90 to 110%, which are acceptable values.

In addition the prediction results using *N*-PLS model were compared with a voltametric method which improved recently in our laboratory [37]. In this method noscapine could be determined in presence of codeine. Prediction ability of the proposed method and voltametric method for determination of noscapine in presence of codeine are compared in Table 7. It must be noticed that no replication and averaging has been performed in *N*-PLS method.

### 4.7. Comparison between PLS and N-PLS models

In order to compare results obtained by *N*-PLS with results from conventional two-way PLS, one dimension of the calibration data

**Table 6**
Prediction results for the spiked syrup drugs.

| Drug | Added ($\times 10^{-5}$ mol $L^{-1}$) | | Total Found ($\times 10^{-5}$ mol $L^{-1}$) | | Recovery (%) | |
|---|---|---|---|---|---|---|
| | Cod.[a] | Nos.[b] | Cod. | Nos. | Cod. | Nos. |
| Expectorant codeine syrup[c] | 0.00 | 0.80 | 1.01 | 7.30 | – | 91.3 |
| | 1.75 | 1.00 | 2.78 | 0.93 | 101.1 | 92.5 |
| | 1.00 | 1.75 | 2.07 | 1.84 | 106.0 | 105.1 |
| | 1.75 | 2.50 | 2.66 | 2.54 | 94.3 | 102.0 |
| | 2.50 | 1.75 | 3.37 | 1.82 | 94.4 | 104.0 |
| Tonin cough syrup[d] | 0.80 | 0.00 | 0.85 | 0.86 | 103.8 | – |
| | 1.75 | 1.00 | 1.90 | 1.89 | 108.6 | 103.0 |
| | 1.00 | 1.75 | 1.01 | 2.58 | 101.0 | 98.3 |
| | 1.75 | 2.50 | 1.69 | 3.27 | 96.6 | 96.4 |
| | 2.50 | 1.75 | 2.52 | 2.69 | 100.8 | 104.6 |
| Windsor syrup[e] | 0.00 | 0.00 | 0.95 | 0.91 | – | – |
| | 1.75 | 1.00 | 2.86 | 1.97 | 109.0 | 105.8 |
| | 1.00 | 1.75 | 1.86 | 2.57 | 91.4 | 95.0 |
| | 2.50 | 1.75 | 3.55 | 2.61 | 103.8 | 97.1 |
| | 1.75 | 2.50 | 2.81 | 3.32 | 106.5 | 96.4 |

[a] Cod: codeine.
[b] Nos: noscapine.
[c] Each 5 mL contains: Codeine phosphate 10 mg, Guaifenesine 100 mg, Phenylpropanolamine HCl 12.5 mg, Sodium saccharine 7.5 mg, Ethanol 150 mg; (Darou Pakhsh Co., Iran).
[d] Each 5 mL contains: Guaifenesin 50 mg and Noscapine HCl 10 mg; (Sato Pharmaceutical Co., Japan).
[e] Each 5 mL contains: Bromhexine HCl 2 mg, Codeine phosphate 10 mg, Ephedrine HCl 4.6 mg, Noscapine HCl 10 mg; (Franklin Pham. Lab. Co., China).

**Table 7**
Comparison between *N*-PLS and voltametric methods for the determination of noscapine in presence of codeine.

| Drug | Noscapine (mg per 5 mL) | Found (mg per 5 mL) | |
|---|---|---|---|
| | | Proposed method | Literature method [37] |
| Windsor Syrup | 10.0 | 9.4 | 9.9 |

**Table 8**
Added and found results of codeine and noscapine in expectorant codeine samples using conventional two way PLS.

| Added ($\times 10^{-5}$ mol L$^{-1}$) | | Total Found ($\times 10^{-5}$ mol L$^{-1}$) | | Recovery (%) | |
|---|---|---|---|---|---|
| Cod. | Nos. | Cod. | Nos | Cod. | Nos |
| 0.00 | 0.80 | 1.19 | 0.65 | – | 81.3 |
| 1.75 | 1.00 | 3.24 | 1.24 | 117.1 | 124.0 |
| 1.00 | 1.75 | 2.62 | 1.52 | 143.0 | 86.9 |
| 1.75 | 2.50 | 3.54 | 2.40 | 134.3 | 96.0 |
| 2.50 | 1.75 | 3.90 | 1.55 | 108.4 | 88.6 |
| No. of Factors | | 3 | 3 | | |

was eliminated, in this way one slice of previous three dimension data (applied for constructing *N*-PLS model) corresponding to 0.08 mol L$^{-1}$ of $H_2SO_4$ (which is optimum concentration for both codeine and noscapine, see Fig. 4) was selected and this data including 16 samples × 1000 times was employed for constructing PLS model. Next number of factors was optimized using RMSECV method for codeine and noscapine as described in section 4.4. Optimum number of factors was three factors for both codeine and noscapine. The predictive ability of the PLS model at optimum number of factors and optimum concentration of $H_2SO_4$ was determined using expectorant codeine syrup samples (Table 8). In this manner previous 3D-data obtained for expectorant codeine samples was converted to two dimension matrix (one slice of data corresponding to 0.08 mol L$^{-1}$ of $H_2SO_4$ was selected) and PLS model applied for simultaneous determination of codeine and noscapine in expectorant codeine samples. As can be seen in Tables 6 and 8, no satisfactory recoveries could be obtained for codeine and noscapine using conventional two-way PLS in compare to *N*-PLS model.

## 5. Conclusion

A simple, rapid, and direct flow injection chemiluminescent (FI-CL) method was introduced for the simultaneous determination of codeine and noscapine using *N*-PLS regression. The concentration of $H_2SO_4$ was selected as one of the variables in the three-way data, because its influence on the CL intensity of the compounds was different. The accuracy of the method was examined by analysis of the spiked cough syrups. The results reveal the ability of the proposed method.

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