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RESEARCH ARTICLE

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Direct determination of reserpine in urine using excitation-emission fluorescence combined with three-way chemometric calibration methodologies

Abstract The concentration of reserpine in urine was directly and quantitatively measured by using the excitation-emission fluorescence (EEM) combined with three-way calibration methodologies. Two calibration methods are based on the alternating trilinear decomposition (ATLD) and the self-weighted alternating trilinear decomposition (SWATLD) algorithms, respectively. These chemometric methodologies have the second-order advantage, which is the ability to get accurate concentration estimates of interested analyte(s) even in the presence of uncalibrated interferences. The satisfactory results on spiked urine samples are obtained, when the component number was chosen to 3 ($N = 3$) for both the methods. This experiment is easily carried out without time-consuming and complicated pretreatment. It has proved that the three-way calibration methodologies based on ATLD and SWATLD can be feasible to directly quantify the medical content of reserpine in urine.

Keywords reserpine, urine, excitation-emission matrix fluorescence, alternating trilinear decomposition, ATLD, self-weighted alternating trilinear decomposition, SWATLD

Reserpine (RSP), also known as serpasil, can affect norepinephrine in the storage and release. Reserpine is extensively used in a variety of pharmaceutical preparations with antihypertensive properties. As a sort of important hypertension drugs, monitoring its concentration in body fluids is quite significant. Many methods for the determination of reserpine have been proposed, in which liquid chromatography and fluorimetry [1,2] are most widely applied. Anderson et al. [3] utilized the three-step liquid-liquid extraction (LLE) sample preparation procedure to determine reserpine in equine plasma using ionspray liquid chromatography-tandem mass spectrometry (LC-MC-MC). Ke et al. [4] developed an improved analytical method employing a divalent cation chelating agent (disodium EDTA) for sample treatment for determination of reserpine in FVB/N mouse plasma with turbo ionspray LC-MS-MS. Clinical studies have shown that about 20% of the eaten reserpine is discharged in urine from the body. However, due to the fact that the fluorescence signals from reserpine overlap seriously those from the urine matrix, these direct spectrofluorimetric determinations of reserpine became much complicated [5].

In the recent decades, three-way chemometric calibration methodologies have been greatly developed and are being increasingly used for data processing in analytical chemistry. Coupled with modern hyphenated instruments such as EEM and liquid chromatography-photodiode array detection (HPLC-DAD), three-way calibration methods with the property of 'mathematical separation' to displace 'chemical separation' is utilized to directly determine single or several component(s) in complex samples or to resolve mixture components, even in the presence of uncalibrated factors. This property has been named the 'second-order advantage' [6]. In this paper, a novel analysis method is proposed to determine the analyte(s) through performing the trilinear decomposition of three-dimensional EEM data array and second-order calibration. Both the alternating trilinear decomposition (ATLD) [7] and the self-weighted alternating trilinear decomposition (SWATLD) [8] algorithms are employed to determine reserpine in complex human urine. The results obtained are satisfactory. The proposed strategy avoids a series of separation procedures and is rapid and low-cost.

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1 Theory

1.1 Trilinear component model

Suppose that a given sample produces an $I \times J$ data matrix or second-order array by EEM, where I represents the number of excitation wavelengths and J is the number of emission wavelengths, respectively. If K samples, consisting of the calibration samples and prediction samples, are stacked, a three-way data array $\underline{\boldsymbol{X}}$ is obtained with $I \times J \times K$ dimensions. This array $\underline{\boldsymbol{X}}$ can be written as:

$$x_{ijk} = \sum_{n=1}^{N} a_{in} b_{jn} c_{kn} + e_{ijk} \quad (i = 1,2,...,I; j = 1,2,...,J; k = 1,2,...,K) \tag{1}$$

where N notes the number of overall factors, which should correspond to the total number of detectable species, including the component(s) of interest and the background as well as potential unknown interference(s); x_{ijk} represents a fluorescence intensity element; a_{in} is the element of an $I \times N$ matrix $\boldsymbol{A}$ corresponding to excitation profiles; b_{jn} is the element of a $J \times N$ matrix $\boldsymbol{B}$ corresponding to emission profiles; c_{kn} is the element of a $K \times N$ matrix $\boldsymbol{C}$ corresponding to relative concentrations of N species; and e_{ijk} is the element from an $I \times J \times K$ three-way residual array $\underline{\boldsymbol{E}}$.

1.2 ATLD algorithm

For the trilinear model Eq. (1), the objective function to be minimized is defined as the sum of the squares of the elements of the residual matrices. According to the cyclic symmetry property of the trilinear model [7], it can be represented in matrix notation as follows:

$$\sigma_1(A) = \sum_{i=1}^{I} \left\| X_{i..} - B\,diag\left(a_{(i)}\right) C^T \right\|_F^2 \tag{2}$$

$$\sigma_2(B) = \sum_{j=1}^{J} \left\| X_{.j.} - C\,diag\left(b_{(j)}\right) A^T \right\|_F^2 \tag{3}$$

$$\sigma_3(C) = \sum_{k=1}^{K} \left\| X_{..k} - A\,diag\left(c_{(k)}\right) B^T \right\|_F^2 \tag{4}$$

Here, $\|\bullet\|_F$ indicates the Frobenius matrix norm.

From the updated $\boldsymbol{A}$, $\boldsymbol{B}$ and $\boldsymbol{C}$ by Eq. (2)–(4) based on the alternating least-squares principle, one can get the relative excitation matrix $\boldsymbol{A}$, the relative emission matrix $\boldsymbol{B}$ and the relative concentration matrix $\boldsymbol{C}$ as follows:

$$a_{(i)}^T = diag\left(B^+ X_{i..} \left(C^T\right)^+\right), i = 1,...,I \tag{5}$$

$$b_{(j)}^T = diag\left(C^+ X_{.j.} \left(A^T\right)^+\right), j = 1,...,J \tag{6}$$

$$c_{(k)}^T = diag\left(A^+ X_{..k} \left(B^T\right)^+\right), k = 1,...,K \tag{7}$$

Using the Moore-Penrose generalized inverses based on singular value decomposition (SVD), the ATLD algorithm has the property of being insensitive to the estimated component numbers and fast convergence.

1.3 SWATLD algorithm

Based on the different error brought in different measurement points, SWATLD [8] algorithm was developed in our laboratory. SWATLD aims to alternately minimize three objective functions with intrinsic relationships on basis of ATLD and calculated $\boldsymbol{C}$, $\boldsymbol{A}$ and $\boldsymbol{B}$ as the following expressions:

$$c_{(k)} = \frac{1}{2}\left(diagm\left(B^+ X_{..k}^T A\right)./diagm\left(A^T A\right) + diagm\left(A^+ X_{..k}^T B\right)./diagm\left(B^T B\right)\right) \quad k = 1,2,...,K \tag{8}$$

$$b_{(j)} = \frac{1}{2}\left(diagm\left(A^+ X_{.j.}^T C\right)./diagm\left(C^T C\right) + diagm\left(C^+ X_{.j.}^T A\right)./diagm\left(A^T A\right)\right) \quad j = 1,2,...,J \tag{9}$$

$$a_{(i)} = \frac{1}{2}\left(diagm\left(C^+ X_{i..}^T B\right)./diagm\left(B^T B\right) + diagm\left(B^+ X_{i..}^T C\right)./diagm\left(C^T C\right)\right) \quad i = 1,2,...,I \tag{10}$$

This algorithm avoids possible "swamp areas" by alternatively minimizing three different objective functions. Similar to ATLD, SWATLD has also the features of fast convergence and being insensitive to the excess factors used in calculation.

After the three-dimensional data array was processed with trilinear decomposition, the detail steps of quantitative analysis for components of interest can be referred to Ref. [7].

2 Experimental

2.1 Reagents and apparatus

Reserpine was purchased from National Institute for Control of Pharmaceutical and Biological Products in

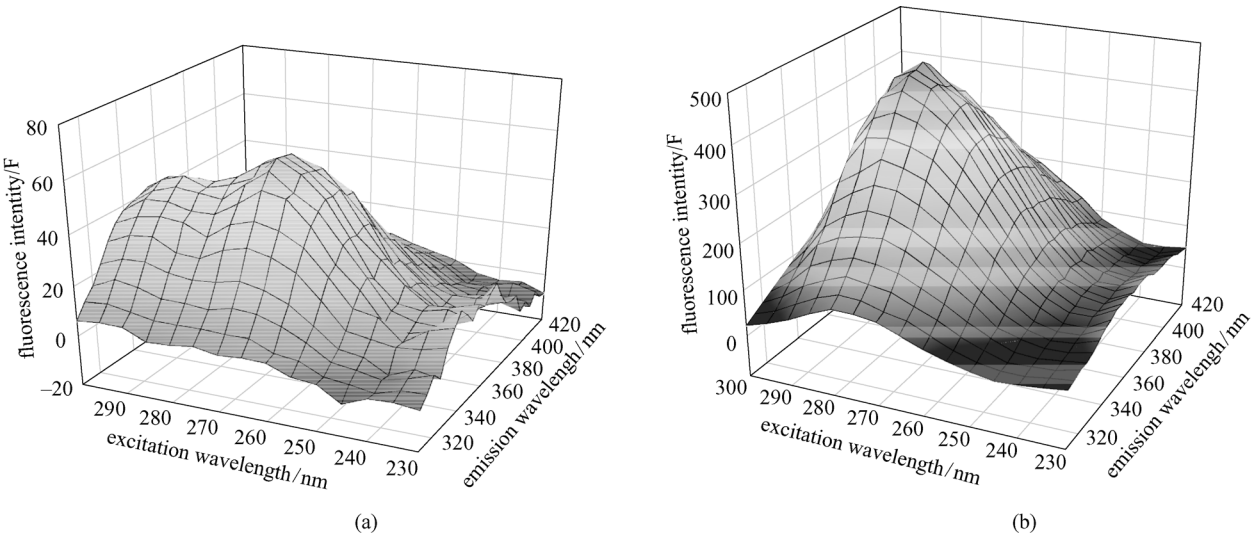

Fig. 1 Excitation-emission fluorescence spectra of pure reserpine (a) and reserpine in urine (b)

Changsha. Acetic acid was of analytical grade. The urine was from the healthy volunteer without eating drugs. Doubly distilled water was used throughout.

All fluorescence measurements were carried out on a Hitachi (Tokyo, Japan) F-4500 fluorescence spectrophotometer equipped with a 150-W Xe lamp. The excitation wavelength was set from 310.0 to 420.0 nm at an interval of 5.0 nm, and the emission wavelength varied from 430.0 to 505.0 nm with an interval of 5.0 nm. Slit width was 5.0 nm/5.0 nm, and scan speed was 1200 nm/min. All computer programs were written in Matlab and all calculations were carried out on a personal computer.

2.2 Analytical methodology

A stock solution of 10 μg/mL reserpine was prepared and stored in the dark at 4°C. All the working solutions with different concentrations were prepared by diluting the standard stock solution. The first four samples only contain reserpine as the calibration set and the last three predicted samples were spiked to human urine as background or interferences. Thus, a $22 \times 15 \times 7$ data array can be assembled. Rayleigh scattering in all response matrices was roughly corrected just by subtracting the average response matrix of the three blank solutions.

3 Results and discussion

3.1 Analysis of fluorescence spectra

Since urine usually exhibites strong fluorescence, the correct number of spectral components in urine warrants investigation. Our experiments confirmed that the biggest excitation and emission wavelength of reserpine were 265 nm and 358 nm. Their fluorescence spectra were heavily overlapped (see Fig. 1), where Figs. 1(a) and 1(b) were three-dimensional fluorescence spectra of pure reserpine and the reserpine in urine with the same concentration ($c = 80$ ng/ml), respectively. It was shown that the fluorescence spectrum of reserpine was almost entirely covered by the fluorescence spectrum in urine. Generally, it is difficult to directly quantify the concentration of reserpine in urine because of their fluorescence selectively.

The response data array obtained from EEM was resolved by second-order calibration methods. Fig. 2 showed plots of the emission and excitation spectral profiles provided by ATLD with $N = 2$ when the urine samples were processed together with the calibration set. It was clear that, in the presence of urine, these ATLD-resolved profiles nicely matched those measured for a pure reserpine solution, meaning that the second-order advantage has been exploited.

3.2 Results for spiked urine samples

The linear range of reserpine concentration was 40–320 ng/mL in this experiment. The correlation coefficients were more than 0.99 and there was satisfying linear relationship.

Both ATLD and SWATLD algorithms were employed to carry out the trilinear decomposition of three-dimensional EEM data array ($22 \times 15 \times 7$) when $N = 2$ and $N = 3$ were tested as the number of factors, respectively. The prediction results for the spiked urine samples were listed in Table 1. As can be seen, the results of ATLD with $N = 2$ were relatively lower than those of other cases, and the three-factor models, compared with two-factor models, offered better predicted values. The possible

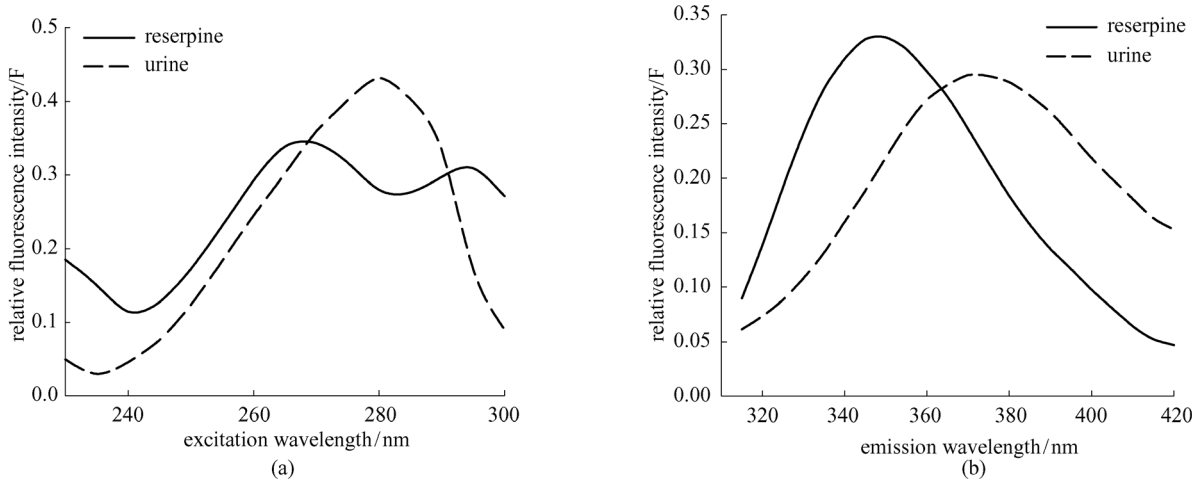

Fig. 2 Resolved spectra corresponding to the reserpine and the urine respectively by using ATLD with $N = 2$: (a) exicitation spectra and (b) emission spectra

Table 1 Determination results of reserpine in urine by using EEM spectra combined with ATLD/SWATLD-based three-way calibrations

predicted sample	actual values/ng·mL^{-1}	found by ATLD/ng·mL^{-1}		found by SWATLD/ng·mL^{-1}	
		$N = 2$[a]	$N = 3$[a]	$N = 2$[a]	$N = 3$[a]
S1	125	112.1(89.7)	117.9(94.3)	118.7(94.8)	120.9(96.7)
S2	160	145.8(91.1)	153.4(95.9)	148.1(92.4)	153.6(96.0)
S3	200	170.6(85.3)	193.5(96.7)	176.3(87.9)	186.0(93.0)
Average recoveries (%)	–	88.7 ± 2.6	95.6 ± 0.9	91.7 ± 2.5	95.2 ± 1.5

[a] The recoveries (%) are indicated in parentheses.

reason for this was that some nonlinear factors were avoided in course of the experiments as the increased component was performed linearity fitting with original measured data. Overall, all the recoveries were satisfactory for the samples of the complicated urine.

Because ATLD and SWATLD were insensitive to the estimated number of components in the model, two-factor models and the three-factor models achieved similar and reasonable results. In Fig. 3, the corresponding concentration profiles of the data obtained by ATLD decomposition were shown. Figs. 3(a) and 3(b) showed the estimated relative concentration profiles with $N = 2$ and with $N = 3$, respectively. It was obvious that the profiles of the two-factor model agreed with two of three profiles (Reserpine and Urine) of the three-factor model. In Fig. 3(b), the third profile was near to 0 in every sample point, which was composed of the background and other interferences without fluorescence.

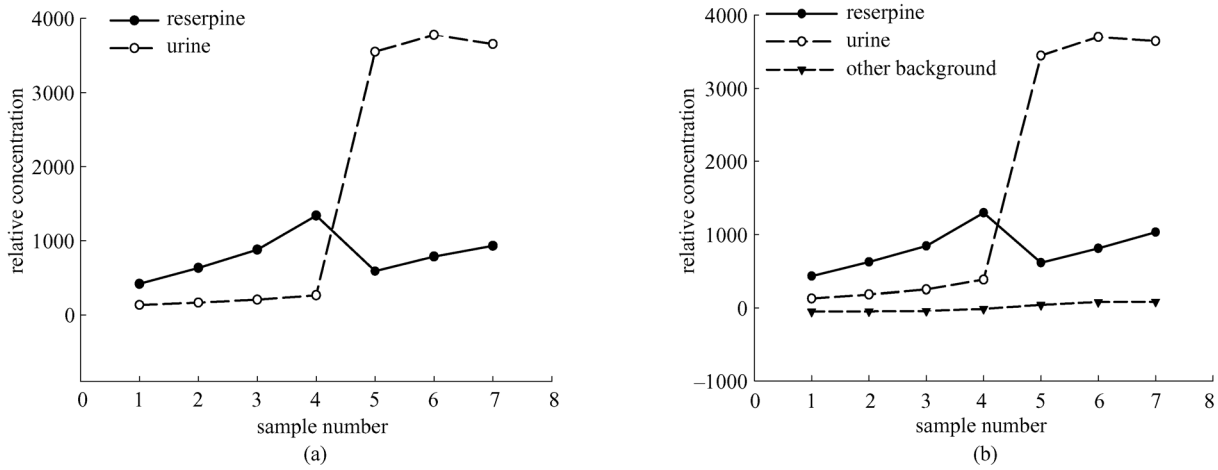

Fig. 3 Resolved concentration profile using ATLD: (a) $N = 2$ and (b) $N = 3$

The obtained results further demonstrated that the three-way calibration methods are excellent tools for the quantification of analyte(s) of interest in complex samples. Moreover, ATLD and SWATLD have the advantage of fast convergence and insensitivity to the component numbers so that the analytical workers don't need cost a great deal of time and energy to do chemical separation and accurately estimate the component numbers of three-dimensional data matrices.

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