Research Article

Micellar Enhanced Three-Dimensional Excitation-Emission Matrix Fluorescence for Rapid Determination of Antihypertensives in Human Plasma with Aid of Second-Order Calibration Methods

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A highly sensitive three-dimensional excitation-emission fluorescence method was proposed to determine antihypertensives including valsartan and amlodipine besylate in human plasma with the aid of second-order calibration methods based on parallel factor analysis (PARAFAC) and alternating trilinear decomposition (ATLD) algorithms. Antihypertensives with weak fluorescent can be transformed into a strong fluorescent property by changing microenvironment in samples using micellar enhanced surfactant. Both the adopted algorithms with second-order advantage can improve the resolution and directly attain antihypertensives concentration even in the presence of potential strong intrinsic fluorescence from human plasma. The satisfactory results can be achieved for valsartan and amlodipine besylate in complicated human plasma. Furthermore, some statistical parameters and figures of merit were evaluated to investigate the performance of the proposed method, and the accuracy and precision of the proposed method were also validated by the elliptical joint confidence region (EJCR) test and repeatability analysis of intraday and interday assay. The proposed method could not only light a new avenue to directly determine valsartan or amlodipine besylate in human plasma, but also hold great potential to be extended as a promising alternative for more practical applications in the determination of weak fluorescent drugs.

1. Introduction

Hypertension is considered as a chronic disease resulting in the elevation of arterial hypertension and increasing the risk of cardiac disease [1]. The relationship between pharmacological reduction in blood pressure for hypertensive individuals and decreasing the risk of suffering cardiovascular and renal complications has been known for more than half century [2]. Valsartan and amlodipine besylate, the representatives of first-line antihypertensive drugs, have been successfully applied to the treatment of resistant hypertension with few adverse effects, robust efficacy, and persistence

antihypertensive effect in the long term [3–5]. Valsartan selectively inhibits the binding of Angiotensin II to the Angiotensin I receptor in many tissues including vascular smooth muscle and the adrenal gland and blocks vasoconstriction as well as aldosterone-secreting effects of Angiotensin II, therefore reducing blood pressure [6–9]. On the other hand, Amlodipine, a derivative of dihydropyridines, blocks calcium influx into vascular smooth muscle and cardiac muscle, leading to a decrease in peripheral vascular resistance [10–12]. In order to further investigate the pharmacokinetics of valsartan and amlodipine, the analytical methods with regard to valsartan and amlodipine

under the background interference of human plasma are urgent to be developed for their significant application value.

Based on the available literature, several analytical methods had been reported for the quantification of valsartan or amlodipine in biological matrixes. Liquid chromatography-tandem mass spectrometry using electrospray ionization mode [13–15], differential-pulse adsorptive stripping and square-wave adsorptive stripping voltammetry [16], High Performance Liquid Chromatography (HPLC) with Diode Array Detector (DAD), and second derivative-spectrophotometric have been applied to the determination of valsartan [17, 18]. In the meantime, for example, Gas Chromatography (GC) [19], online coupled isotachopheresis-capillary zone electrophoresis with Diode Array Detector (DAD) [20–22], HPLC with UV or fluorescence detection (FLD), and tandem mass spectrometric (MS/MS) were applied for the quantification of amlodipine [23, 24]. However, the GC methods may cause the thermal decomposition of analytes at high temperatures. As for capillary electrophoresis methods, the changes in sample composition are responsible for the changes in electroosmosis, which correspondingly influence the separation repeatability. Unfortunately, HPLC methods are disadvantaged by high volumes of toxic solvents and its separation procedure is time-consuming; what is more, researchers have to develop an extremely complex gradient for the separation and specific preprocessing procedures for samples, which is often a painful work to achieve.

Compared with the conventional fluorescence spectrometry, chemometric methodologies coupled with excitation-emission matrix (EEM) fluorescence can enhance the selectivity of analytical methods and reduce analytical cost. The predominant advantage of second-order calibration is that it allows concentration information of an individual component to be extracted even in the presence of uncalibrated interferences, and besides this method presented satisfactory results and had advantages over other conventional methods [25, 26]. To the extent of our literature search, the determination of valsartan or amlodipine in human plasma by a spectrofluorimetric method combined with second-order calibration has not been reported so far.

In this study, a simple, rapid, and effective method for the direct quantitative analysis of valsartan or amlodipine in human plasma by means of their strong fluorescence property after micellar enhanced microenvironment in samples was proposed by combining three-dimensional excitation-emission matrix fluorescence technology with second-order calibration strategies based on both PARAFAC [27] and ATLD algorithms [28]. Several statistical parameters such as the figures of merit (FOM) involving sensitivity (SEN), selectivity (SEL), and limit of detection (LOD) were investigated, the precision and accuracy of intraday and interday were calculated, and the reliability of the proposed method was also estimated by using the elliptical joint confidence region (EJCR) test. The results indicate that this method is simple, accurate, and reliable.

2. Materials and Methods

2.1. Apparatus. The measurements of fluorescence were performed with a HITACHI F-7000 Fluorescence spectrophotometer fitted with a xenon lamp and interfaced to a Dell PowerEdge T420. In all cases, 1.00 cm quartz cell was used. The spectra data were imported to computer and analyzed in the Matlab environment. The programs of the PARAFAC and ATLD algorithms were homemade. The room temperature was controlled at 25°C.

2.2. Reagents. Valsartan was purchased from TCI (Shanghai, China); Amlodipine Besylate was bought from National Institutes for Food and Drug Control. Phosphoric acid, sodium dihydrogen phosphate, acetic acid, sodium acetate, and sodium dodecyl sulfate (SDS) of analytical grade were obtained from Sinopharm Chemical Reagent Co., Ltd. The human plasma was obtained from Wuhan Institute of Biological Product Co., Ltd.

Stock solutions of 0.501 mg/mL valsartan and 0.402 mg/mL amlodipine besylate were prepared by dissolving appropriate amount in methanol. Working solutions were prepared by appropriate dilution. The concentration of valsartan and amlodipine besylate in working solution is 5.01 µg/mL and 120.6 µg/mL, respectively. Phosphoric acid/sodium dihydrogen phosphate buffer solution of pH 2.0 and acetic acid/sodium acetate buffer solution of pH 4.0 were employed. The stock solution with 0.1 mol L⁻¹ sodium dodecyl sulfate (SDS) was also prepared. Those solutions were spectrophotometrically stable when protected from light and kept in the refrigerator at 4°C.

2.3. Trilinear Model for Second-Order Calibration. In the case of EEM fluorescence, a three-dimensional data array $\mathbf{X}$, with dimensions of $I \times J \times K$ (I is the number of excitation wavelengths, J is the number of emission wavelengths, and K is the number of samples) can be produced by stacking a series of excitation-emission matrix fluorescence spectra obtained for each of the K samples. The trilinear component model has the following form:

$$x_{ijk} = \sum_{n=1}^N a_{in} b_{jn} c_{kn} + e_{ijk} \quad (1)$$

$$(i = 1, 2, \dots, I; j = 1, 2, \dots, J; k = 1, 2, \dots, K),$$

where N denotes the total number of fluorescing species, which includes the components of interest, background, and uncalibrated interferences. x_{ijk} represents the fluorescent intensity of sample k at excitation wavelengths i and emission wavelengths j . c_{kn} is the element (k, n) of an $K \times N$ matrix $\mathbf{C}$ with relative concentrations of the N species in K samples. a_{in} is the element (i, n) of an $I \times N$ matrix $\mathbf{A}$ with relative excitation spectra of the N species. b_{jn} is the element (j, n) of an $J \times N$ matrix $\mathbf{B}$ with relative emission spectra of the N species, and e_{ijk} is the element of the three-way residual array $\mathbf{E}$ ($I \times J \times K$).

2.4. PARAFAC and ATLD Algorithms. The PARAFAC model proposed by Harshman, Carroll, and Chang is known as the canonical decomposition, which has been accepted owing to its consistency with Beers law in chemistry. This algorithm is based on a least-squares minimization. The second-order calibration algorithm based on PARAFAC, which is used widely in chemistry for its excellent performance, was adopted to resolve the overlapped spectroscopes and to give a satisfying result. However, the application of PARAFAC may be obstructed by the requirement of a precise estimation of the number of components in the mixtures and characteristic of slow convergence rate.

An improved algorithm, namely, alternating trilinear decomposition (ATLD), was proposed by Wu et al. to overcome the aforementioned disadvantages. It uses the alternating least-squares principle, Moore-Penrose generalized inverse based on singular value decomposition (SVD), and alternating iterative strategy to improve the performance of trilinear decomposition, making a loss function to reach a minimum. In general, ATLD shows a faster convergence than PARAFAC and eliminates the uncertainty associated with factor number estimation.

2.5. Figures of Merit. Figures of merit (FOM) including SEN, SEL, LOD, and LOQ are frequently used to validate the results and compare the performance of various methods. In second-order calibration, the evaluations of FOM are closely related to the calculation of the net analyte signal (NAS), which is defined as the part of the signal that relates uniquely to the NAS. The SEN is estimated as the NAS at unit concentration, and the SEL is the ratio between the sensitivity and the total signal. The LOD of one method is the lowest quantity of a substance that can be distinguished from the absence of that substance (a background value) within a stated confidence limit. The LOQ is the limit at which we can reasonably tell the difference between two different values. Those formulas of FOM are estimated as

$$\begin{aligned} \text{SEN} &= \lambda \left\{ \left[(\mathbf{A}^T \mathbf{A})^{-1} \right] \times \left[(\mathbf{B}^T \mathbf{B})^{-1} \right] \right\}_{nn}^{-1/2} \\ \text{SEL} &= \left\{ \left[(\mathbf{A}^T \mathbf{A})^{-1} \right] \times \left[(\mathbf{B}^T \mathbf{B})^{-1/2} \right] \right\}_{nn}^{-1/2} \\ \text{LOD} &= 3.3s(0) \\ \text{LOQ} &= 10s(0), \end{aligned} \quad (2)$$

where nn means the (n, n) element of matrix $[(\mathbf{A}^T \mathbf{A})^{-1} \times (\mathbf{B}^T \mathbf{B})^{-1}]$, λ is the total signal for component n at unit concentration, and the symbol $\times$ indicates the Hadamard product. $s(0)$ is the standard deviation in the predicted concentration for three different background blank samples, in the algorithms.

The root-mean-square error of prediction (RMSEP) can be calculated in terms of the formula as $\text{RMSEP} = [(I/(I - 1)) \sum_{i=1}^I (C_{\text{act}} - C_{\text{pred}})^2]^{1/2}$, where I is the number of prediction samples and C_{act} and C_{pred} are the actual and predicted concentrations of the analytes, respectively.

2.6. Analytical Methodology. As for valsartan, Twenty-seven samples including eleven calibration samples, six test samples, and ten prediction samples were prepared with concentrations within the range of 78.2–559.1 ng/mL. Calibration samples and test samples were made by appropriate dilution of the analyte working solution in methanol. Ten prediction samples contained ten different concentrations of valsartan, 0.01 mol L⁻¹ of SDS, phosphoric acid/sodium dihydrogen phosphate buffer solution (PH = 2.0), and 10 μ L human plasma. The concentrations of each component in the samples are given in Table 1.

As regards amlodipine besylate, twenty-five samples including eleven calibration samples, six test samples, and eight prediction samples were prepared with concentrations within the range of 1.93–12.06 μ g/mL. Calibration samples and test samples were made by appropriate dilution of the analyte working solution in methanol. Eight prediction samples contained different concentrations of amlodipine besylate, 0.01 mol L⁻¹ of SDS, acetic acid/sodium acetate buffer solution (PH = 4.0), and 10 μ L human plasma. The concentrations of each component in the samples are also given in Table 1.

2.7. Parameters. As for valsartan, in order to avoid the Rayleigh and Raman scatterings, all the spectral surfaces were recorded at excitation wavelengths varying from 200 to 292 nm at a 2 nm step and emission wavelengths varying from 310 to 378 nm at a 2 nm step. For the same reason, all the spectral surfaces of amlodipine besylate were recorded at excitation wavelengths varying from 266 to 320 nm at a 2 nm step and emission wavelengths varying from 374 to 440 nm at a 2 nm step. The slit width was 10.0/10.0 nm and the scan rate was 2400 nm/min. Thus, a $47 \times 35 \times 27$ (excitation wavelength $\times$ emission wavelength $\times$ samples) data array for valsartan and a $28 \times 34 \times 25$ (excitation wavelength $\times$ emission wavelength $\times$ samples) data array for amlodipine besylate were assembled.

3. Results and Discussion

3.1. Fluorescence Spectrum of Valsartan and Amlodipine Besylate. To investigate the fluorescence properties of the valsartan and amlodipine besylate, the pure valsartan and amlodipine besylate were prepared and measured via fluorescence spectrophotometer at the given parameters; Figures 1(a) and 2(a) show valsartan and amlodipine besylate with weak fluorescence, which is hard to determine at the low concentration due to matrix effect, therefore requiring researchers to adopt possible ways, namely, micellar enhanced spectrofluorometric method to increase the fluorescence of two analytes; thus SDS, a kind of surfactant, is to stabilize and enhance the fluorescence intensity of both valsartan and amlodipine besylate; it can be safely observed that the fluorescence intensity of both analytes has remarkably increased as shown in Figures 1(b) and 2(b). In this work, it is necessary to measure the fluorescence intensity of human plasma because all the prediction samples are assayed at the background interference of human plasma; Figures 1(c) and 2(c) show that

TABLE 1: The concentration of valsartan and amlodipine besylate in calibration, test, and prediction samples.

Analytes	Calibration samples		Test samples		Prediction samples	
	Number	C (ng/mL)	Number	C (ng/mL)	Number	C (ng/mL)
Valsartan	1	78.2	12	180.4	18	90.2
	2	126.3	13	240.5	19	138.3
	3	174.3	14	300.6	20	186.4
	4	222.4	15	360.7	21	234.5
	5	270.5	16	420.8	22	282.6
	6	318.6	17	481.0	23	330.7
	7	366.7			24	378.8
	8	414.8			25	426.9
	9	462.9			26	474.9
	10	511.0			27	523.0
	11	559.1				
Amlodipine besylate	28	1.93	39	3.02	45	3.38
	29	2.95	40	4.22	46	4.46
	30	3.98	41	5.43	47	5.55
	31	5.00	42	6.63	48	6.63
	32	6.03	43	7.84	49	7.72
	33	7.06	44	9.05	50	8.80
	34	8.08			51	9.89
	35	9.11			52	10.97
	36	10.13				
	37	11.16				
	38	12.06				

the three-dimensional fluorescence spectra of two analytes which were heavily overlapped with human plasma contained intense inherent fluorescence. The three-dimensional fluorescence spectra about two analytes are shown in Figures 1 and 2.

However, a serious profile overlapping between the valsartan and human plasma together with amlodipine besylate and human plasma was experimentally observed, which consequently made the quantitative analysis of the two analytes using traditional fluorescent methodologies impossible; one can resort to the second-order calibration methods which allow for unique decomposition of trilinear data and only require that the species of interest in both calibration samples and the prediction samples are the same, so with the aid of chemometrics the interference of human plasma can be mathematically separated and accomplish reliable resolution of spectra and accurate quantification of valsartan and amlodipine besylate in complicated biological matrix.

3.2. Establishment and Validation of Two Calibration Models.

Accurate and reliable calibration models are the key for direct determination of either valsartan or amlodipine besylate with the interference of human plasma; the reliability of the two calibration models for valsartan samples and amlodipine besylate was validated by average recovery and different statistical parameters. The results were shown in Table 2; the average recoveries of valsartan or amlodipine besylate in the six test samples were found to be $100.3 \pm 1.3\%$ and $99.3 \pm 3.3\%$; the RMSEP was calculated to be 3.64 ng/mL

and 0.27 $\mu\text{g/mL}$, respectively. Student's t -test illustrates that the establishment of experiment models possesses statistical significance and the results clearly indicate that the chosen chemometric algorithms are reliable for the quantification of valsartan or amlodipine besylate in human plasma.

3.3. Estimation of Component Numbers. The correct analytical results can hardly be obtained when the number of components is either big or small; hence it is crucial that the estimation of component numbers is close to the actual one for the purpose of the accurate resolution and validation result; therefore, the core consistency diagnostic (CORCONDIA) as a classical estimation method is applied for solving such a frustrating problem. CORCONDIA was proposed by Bro and Kiers and used to estimate the correct component numbers before the concentration prediction of valsartan or amlodipine besylate in human plasma. The definition of function is as follows:

core consistency

$$= 100 \left[1 - \frac{\sum_{i=1}^F \sum_{j=1}^F \sum_{k=1}^F (g_{ijk} - t_{ijk})^2}{\sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K t_{ijk}^2} \right] \quad (3)$$

When the selected number is bigger than the correct factor number, the core consistency is close to zero, even negative. Only when the selected number is equal to or smaller than the correct number, the core consistency is close to one. It is generally acknowledged that the number is equal

FIGURE 1: Three-dimensional plots of the excitation-emission matrix fluorescence spectra: (a) 318.6 ng/mL pure valsartan; (b) 318.6 ng/mL valsartan and 50 μ L SDS; (c) 318.6 ng/mL valsartan and 50 μ L SDS in human plasma; (d) blank human plasma.

to or smaller than the correct number when the value is bigger than 0.5. The values of core consistency at different numbers of factors for valsartan and amlodipine besylate are shown in Figure 3. When the number of factors was less than or equal to 2, the values of core consistency for valsartan were near 1, but when the number exceeded 2, the values reduced drastically even to zero, suggesting that the model of such a system is very trilinear when the number is 2. Thus, the right number of factors should be 2 for valsartan; the component numbers of amlodipine besylate could be estimated as 2 by the same way.

3.4. Determination of Valsartan and Amlodipine Besylate in Human Plasma. The algorithms of PARAFAC and ATLD were employed for the resolution of three-dimensional fluorescent data of both valsartan and amlodipine besylate in human plasma with the optimal factor number of two

($N = 2$); the actual spectral profiles and the loadings from the decomposition of the EEM fluorescence data array were obtained with the help of chemometrics; the actual and resolved excitation-emission spectra were shown in Figures 4 and 5; it was found that not only the loadings in the excitation and emission modes of valsartan and amlodipine besylate were quite similar to its actual ones, but also the structures of the excitation and emission modes of the interesting analyte were not affected by the chosen algorithms, which implies the good reliability and stability of the decomposition based on the second-order methods. These results further confirm that the proposed second-order methods in this paper allow the spectral profiles of analytes of interest to be extracted reliably and accurately even in complex matrices, mainly attributed to the characteristic of trilinear data.

The prediction results about valsartan and amlodipine besylate in human plasma using both PARAFAC and ATLD algorithms were listed in Tables 3 and 4; it indicated

FIGURE 2: Three-dimensional plots of the excitation-emission matrix fluorescence spectra: (a) 9.11 $\mu\text{g/mL}$ pure amlodipine besylate; (b) 9.11 $\mu\text{g/mL}$ amlodipine besylate and 50 μL SDS; (c) 9.11 $\mu\text{g/mL}$ amlodipine besylate and 50 μL SDS in human plasma; (d) blank human plasma.

TABLE 2: Determination of valsartan and amlodipine besylate in test samples using two algorithms.

Analytes	Test samples	12	13	14	15	16	17
Valsartan	Recovery (%)	97.8	100.6	101.4	100.9	100.9	100.2
	Average recovery			100.3 $\pm$ 1.3			
	RMSEP (ng/mL)			3.64			
	$T(t\text{-test})t_{0.01}^5 = 3.37$			$0.64 < t_{0.01}^5$			
Amlodipine besylate	Test samples	39	40	41	42	43	44
	Recovery (%)	99.5	103.4	99.4	102.1	97.2	94.3
	Average recovery			99.3 $\pm$ 3.3			
	RMSEP (ng/mL)			0.27			
	$T(t\text{-test})t_{0.01}^5 = 3.37$			$0.50 < t_{0.01}^5$			

that satisfying and reasonable experimental results can be gained utilizing the knowledge of chemometrics. It deserves to be mentioned, especially, that t -test was performed to compare the recoveries of valsartan and amlodipine besylate with the ideal value of 100%. The null hypothesis is “the average recovery is equal to 100%.” Degrees of freedom n are 9 and confidence level is 99%; for valsartan,

$T = 1.15 < t_{0.01}^9$ with PARAFAC and $T = 2.63 < t_{0.01}^9$ with ATLD, which demonstrates that a satisfactory prediction capacity has been acquired by the application of these two algorithms. However, when degrees of freedom n are 7 and confidence level is 99%, for amlodipine besylate, $T = 1.25 < t_{0.01}^7$ with the algorithm of ATLD, which shows that there is no significant difference among the prediction results with

FIGURE 3: The core consistency value about valsartan (a) and amlodipine besylate (b) in human plasma.

FIGURE 4: Actual and resolved excitation spectra (a1) and emission spectra (b1) of valsartan in human plasma using PARAFAC, and actual and resolved excitation spectra (a2) and emission spectra (b2) of valsartan in human plasma using ATLD. The solid lines, dotted solid lines, and dotted lines represented the loadings of valsartan, the loadings for an inherent interference from the human plasma, and the actual spectral profiles of valsartan, respectively.

FIGURE 5: Actual and resolved excitation spectra (a1) and emission spectra (b1) of 78 amlodipine besylate in human plasma using PARAFAC, and actual and resolved excitation spectra (a2) and emission spectra (b2) of valsartan in human plasma using ATL D. The solid lines, dotted solid lines, and dotted lines represented the loadings of amlodipine besylate, loadings for an inherent interference from the human plasma, and the actual spectral profiles of amlodipine besylate, respectively.

TABLE 3: Determination of valsartan in human plasma using PARAFAC and ATL D.

Prediction samples	Actual concentration (ng/mL)	Recovery (%)	
		PARAFAC	ATLD
18	90.2	99.3	96.8
19	138.3	100.8	101.7
20	186.4	100.6	102.2
21	234.5	102.1	103.7
22	282.6	99.2	101.5
23	330.7	98.1	100.9
24	378.8	100.4	103.3
25	426.9	98.9	102.2
26	474.9	97.7	101.8
27	523.0	97.5	101.2
Correlation coefficient		0.9996	0.9996
Regression equation		$Y = -6.67X + 0.0819$	$Y = -3.04X + 0.0820$
Average recovery (%)		99.5 ± 1.5	101.5 ± 1.9
RMSEP (ng/mL)		6.55	7.37
$T(t\text{-test})_{0.01}^9 = 2.28$		$1.15 < t_{0.01}^9$	$2.63 < t_{0.01}^9$

TABLE 4: Determination of amlodipine besylate in human plasma using PARAFAC and ATLD.

Prediction samples	Actual concentration (ng/mL)	Recovery (%)	
		PARAFAC	ATLD
45	3.38	98.8	104.5
46	4.46	107.3	102.2
47	5.55	104.8	100.6
48	6.63	102.7	98.2
49	7.72	108.1	103.9
50	8.80	109.1	105.6
51	9.89	103.1	99.7
52	10.97	98.8	96.6
Correlation coefficient		0.9976	0.9971
Regression equation		$Y = -23.9X + 0.028$	$Y = -55.7X + 0.027$
Average recovery (%)		104.2 ± 3.8	101.4 ± 3.2
RMSEP (ug/mL)		0.44	0.24
$T(t\text{-test})t_{0.01}^7 = 2.99$		$3.13 > t_{0.01}^7$	$1.25 < t_{0.01}^7$

TABLE 5: Figures of merit of valsartan and amlodipine besylate in human plasma using two algorithms.

Figures of merit	Valsartan		Amlodipine besylate	
	PARAFAC	ATLD	PARAFAC	ATLD
SEN (mL/ng)	3.23	3.23	0.022	0.066
SEL	0.44	0.44	0.05	0.15
LOD (ng/mL)	0.052	0.11	0.044	0.046
LOQ (ng/mL)	0.61	0.34	0.13	0.14

ATLD. Unfortunately, for amlodipine besylate, $T = 3.13 > t_{0.01}^7$ with the algorithm of PARAFAC, so the recovery is significantly different from 100%. In summary, the algorithm of ATLD has a better capacity to directly determine the concentration of amlodipine besylate in complex human plasma compared with the algorithm of PARAFAC.

3.5. Figures of Merit. Due to the fact that the chosen algorithms have different performance, it is vital to set up a criteria such as FOM for the validation of results and the comparison of various algorithms. In this work, FOM including SEN, SEL, LOD, and LOQ were calculated to compare the performance of two algorithms; the results were shown in Table 5. One can find that the proposed second-order calibration method based on both PARAFAC and ATLD can yield satisfactory predictive results for quantitative analysis of either valsartan or amlodipine besylate in the human plasma.

For the sake of a further investigation into the accuracy of these algorithms, the actual concentrations were linearly regressed against the predicted concentrations. The calculated intercept and slope were compared with their ideal values of 0 and 1, based on the elliptical joint confidence region (EJCR) test. Figure 6 gives the results of EJCRs for PARAFAC and ATLD. It demonstrates that the ideal point (0, 1) signed with a pentacle (★) lies in the EJCRs, and the elliptic size corresponding to the PARAFAC algorithm is smaller than those related to the ATLD algorithms, which

means that the performance of PARAFAC is slightly better than those of ATLD in such systems. These factors further prove that all the algorithms can give precise results in complex system.

3.6. Accuracy and Precision. In order to validate the accuracy and precision of the proposed method for two analytes, our group randomly selected Number 22 (valsartan) and Number 48 (amlodipine besylate) prediction sample, which was prepared in triplicate, and each of them was repetitively measured three times in a day and lasted for three consecutive days; the corresponding results were listed in Table 6. As is shown in Table 6, it can be easily observed that both intraday and interday relative standard deviation of concentrations of valsartan with the algorithms of PARAFAC and ATLD are both less than 1%, which demonstrate that the proposed method are considered to be accurate and precise for the direct determination of valsartan in human plasma. As for amlodipine besylate, the intraday and interday relative standard deviation of concentrations of amlodipine besylate with ATLD are smaller than with PARAFAC, which speak volumes for the fact that ATLD has a relatively higher precision and better repeatability when compared with the algorithm of PARAFAC.

4. Conclusions

In this paper, excitation-emission (EEM) fluorescence data matrix was processed by PARAFAC and ATLD algorithms for the determination of valsartan and amlodipine besylate in human plasma with intense intrinsic fluorescence. Herein, the second-order advantage was adequately exploited in the data mining, which would help obtain quantitative information of two antihypertensives in the presence of uncalibrated interferences, namely, human plasma. Furthermore, after the evaluation of a series of indexes, including the FOM, the EJCR tests, and the accuracy and precision of the proposed method, it can be further confirmed that both algorithms could give

FIGURE 6: EJCRs for valsartan (a) and amlodipine besylate (b) by applying PARAFAC and ATLD with $N = 2$. The pentacle (★) indicates the ideal points (0, 1). The solid lines and dotted lines correspond to the EJCRs in human plasma by applying ATLD and PARAFAC, respectively.

TABLE 6: Intra- and interaccuracy and precision of valsartan and amlodipine besylate using two algorithms.

Analytes	Algorithms	Intraday		Interday	
		Mean $\pm$ SD ^a	RSD ^b %	Mean $\pm$ SD ^a	RSD ^b %
Valsartan	PARAFAC	279.8 $\pm$ 2.6	0.94	279.4 $\pm$ 2.0	0.72
	ATLD	283.6 $\pm$ 2.2	0.76	283.8 $\pm$ 2.1	0.73
Amlodipine besylate	PARAFAC	6.82 $\pm$ 0.01	0.96	6.86 $\pm$ 0.02	2.9
	ATLD	6.67 $\pm$ 0.01	0.35	6.74 $\pm$ 0.01	2.1

^arefers to standard deviation, ^brefers to relative standard deviation.

accurate results for two analytes; however, the performance of ATLD was proved to be better than that of PARAFAC in the cases suffering from matrix effects. This proposed scheme with aid of second-order calibration algorithm could not only provide a novel, rapid, and reliable reference method for the analysis of biological samples, but also possess great potential to be further tailored as a general and promising alternative for the determination of weak fluorescent drugs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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