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Simultaneous Determination of Free Amino Acid Content in Tea Infusions by Using High-Performance Liquid Chromatography with Fluorescence Detection Coupled with Alternating Penalty Trilinear Decomposition Algorithm

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ABSTRACT: In this paper, a novel application of alternating penalty trilinear decomposition (APTLD) for high-performance liquid chromatography with fluorescence detection (HPLC-FLD) has been developed to simultaneously determine the contents of free amino acids in tea. Although the spectra of amino acid derivatives were similar and a large number of water-soluble compounds are coextracted, APTLD could predict the accurate concentrations together with reasonable resolution of chromatographic and spectral profiles for the amino acids of interest owing to its "second-order advantage". An additional advantage of the proposed method is lower cost than traditional methods. The results indicate that it is an attractive alternative strategy for the routine resolution and quantification of amino acids in the presence of unknown interferences or when complete separation is not easily achieved.

KEYWORDS: tea, amino acids, high-performance liquid chromatography with fluorescence detection (HPLC-FLD), second-order calibration, alternating penalty trilinear decomposition algorithm (APTLD)

INTRODUCTION

Tea is beneficial to human health due to the presence of compounds such as polyphenols, amino acids, vitamins, carbohydrates, caffeine, and purine alkaloids. The determination of the polyphenol and caffeine contents and their physiological activities have been widely studied in past decades.[1−3] Tea also contains abundant amino acids with amounts of >1% in dry weight tea. Various studies have demonstrated that amino acids also play an important role in the characteristic flavor and delicate taste of tea.[4−6] Up to now, more than 26 amino acids have been found in tea, including 20 basal amino acids and 6 nonprotein amino acids. Besides the 20 basal amino acids, 6 nonproteinogenic amino acids consist of theanine (Thea), γ-aminobutyric acid (GABA), etc. Of these amino acids, 8 are histidine, lysine, phenylalanine, methionine, leucine, isoleucine, valine, and threonine, which are considered to be more essential to the human diet for proper functioning.[7,8] Thea is a unique free amino acid found almost exclusively in tea plants. It is the main component responsible for giving the tea taste, with an amount of 50% of total amino acid content. Furthermore, it possesses some biological activities, such as taking part in the biosynthesis of polyphenols,[9] decreasing the level of norepinephrine and serotonin in the brain and lowering the blood pressure,[10] benefiting antitumor activity,[11] and so on. GABA, which is the main inhibitory amino acid neurotransmitter in the mammalian central nervous system, has been found to be associated with a decline in blood pressure with continuous drinking of anaerobically treated tea.[12] Amino acids are indispensable nutritional elements for the human body and are involved in many biological activities such as preventing disease, promoting relaxation, inhibiting caffeine's negative effects, reducing blood pressure, and enhancing antitumor activity.

Due to the biological significance of amino acids contained in tea, various analytical methods have been proposed including capillary electrophoresis,[13,14] ion-exchange chromatography,[15] high-performance chromatography,[16−18] etc. Traditionally, the determination of amino acids has been conducted by ion-exchange chromatography followed by postcolumn derivatization with ninhydrin, for which special automatic commercial apparatuses have been created. In recent years, the precolumn derivatization with reversed-phase high-performance liquid chromatography (RP-HPLC) separation has become widely accepted for amino acid analysis. This approach requires much shorter analysis times and gives greater sensitivity than the ion-exchange chromatography method. Among various derivatization reagents, *O*-phthalaldehyde (OPA) is widely used because its derivatization is rapid even when conducted at ambient temperature and the resultant derivatives are very stable and highly fluorescent,[19] although other reagents such as phenylisothiocyanate for UV detection have also been proposed.

Generally, tea infusion is usually prepared using distilled water, which yields a >50% lixiviating rate; in other words, a large number of other water-soluble extracts are coextracted with the free amino acids such as catechins, caffeine, organic acids, and vitamins. Thus, the complex matrices of the tea infusions have affected the derivatization or separation of the amino acids. To reduce the interference of sample matrix, two categories of strategies are commonly used: one is to perform sample cleanup before injection to the analyzer by

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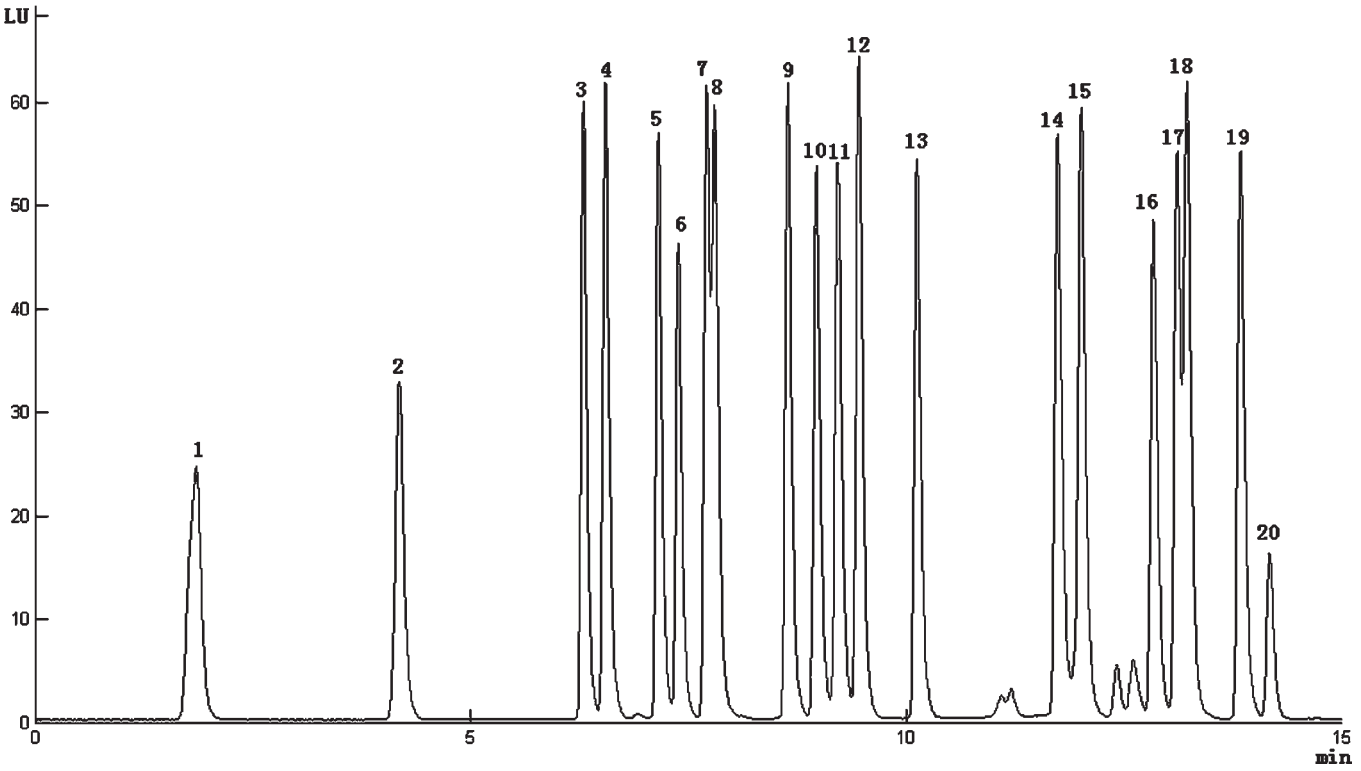

Figure 1. Chromatogram profiles of 20 amino acids derivatized with OPA recorded at Ex 230 nm and Em 455 nm. Peaks: 1, Asp; 2, Glu; 3, Asn; 4, Ser; 5, Gln; 6, His; 7, Gly; 8, Thr; 9, Arg; 10, Ala; 11, GABA; 12, Thea; 13, Tyr; 14, Val; 15, Met; 16, Trp; 17, Phe; 18, Ile; 19, Leu; and 20, Lys.

liquid−liquid extraction purification or solid phase extraction (SPE) steps;[16] the other is to carefully optimize the chromatography conditions to fulfill the complete separation between the target and interfering compounds.[17,18] Unfortunately, both are time-consuming and expensive and require more experienced analysts.

In recent years, a growing number of second-order calibration methods have been proposed to solve the peak overlap in chromatographic separation, especially for highly complex samples. In contrast to traditional measurement techniques, these methods provide a useful resource for accurate quantification of the analytes of interest even in the presence of uncalibrated interference, which is known as "second-order advantage".[20] In recent decades, several algorithms involving the second-order advantage have been proposed and successfully applied for many practical analytical problems, such as the generalized rank annihilation method (GRAM),[21] parallel factor analysis (PARAFAC),[22] multivariate curve resolution−alternating least-squares (MCR-ALS),[23] alternating trilinear decomposition (ATLD),[24] and alternating penalty trilinear decomposition (APTLD).[25]

To our knowledge, simultaneous quantitative analysis of various amino acids with the aid of the second-order calibration method in HPLC analysis has not been reported in previous works. In this paper, second-order data were obtained through HPLC-FLD by set excitation wavelength at 340 nm. Each data point recorded in each run is a function of two parameters, that is, retention time and emission wavelength. Different chromatographic runs produced a three-way array in which the sample number is the third dimension. Then the APTLD algorithm was applied for its deconvolution, integrating a time segment strategy. The results showed the chromatographic and spectral profiles of individual analytes were free from interference of sample matrix and the concentration values were estimated approvingly.

MATERIALS AND METHODS

Chemicals and Samples. Milli-Q water (Millipore, Bedford, MA) was used in all work. HPLC grade acetonitrile and methanol were obtained from Sigma-Aldrich (St. Louis, MO). Most standards of amino acids were also obtained from Sigma-Aldrich; theanine standard was purchased from Tokyo Kasei (Tokyo, Japan). The derivatization reagents OPA and borate buffer were obtained from Agilent (Agilent Technologies, Palo Alto, CA). Other reagents used were of analytical grade. All teas were purchased from local retail shops.

HPLC Analysis. The determination of amino acid content was performed on an Agilent 1200SL HPLC system equipped with a vacuum degasser, an autosampler, a binary pump, a thermostated column oven, and a fluorescence detector (FLD). The separation was completed on a Zorbax Eclipse plus-C18 column (150 × 4.6 mm, 3.5 μm, Agilent). The operating temperature was maintained at 40 °C. Precolumn derivatization with OPA was recommended by Agilent. The mobile phase consisted of phosphate buffer (pH 7.8, A) and methanol/acetonitrile/water (45:45:10, B). The simple gradient elution method was performed as follows: between 0 and 2 min, maintained at 100% of solvent A; 2−16 min, linear gradient change to 50% of solvent A. The flow rate was 2.0 mL/min. The excitation wavelengths of fluorescence were set at 340 nm, and the emission wavelengths were acquired over the range of 365−495 nm with intervals of 5 nm. All of the operations and the acquiring of data were controlled by an Agilent ChemStation.

Calibration Standards. Stock solutions were prepared in 0.1 M HCl, and Asn, Gln, Trp, Thea, and GABA were in deionized water. The mixed working standard solutions were diluted with water daily. A calibration set of 10 samples was constructed. For the simplicity of solution preparation, the concentration level of each analyte was designed approximately as follows: 0, 1, 2, 5, 10, 20, 50, 100, 200, and 250pmol/μL. The orders of these values were randomly arranged to avoid interference between analytes. In addition, to verify the performance of the APTLD algorithm in the system, the validation set was also built with different concentrations

of analytes within their corresponding linear concentration range without interferents added. Duplicate analyses were performed for each sample.

Sample Preparation. Aqueous infusions of teas were used as the source of amino acid analysis. Half a gram of the tea powders was extracted in 100 mL of distilled water at 100 °C for 20 min and then filtered through a "double-ring" no. 102 filter paper (Xinhua Paper Industry Co. Ltd., Hangzhou, China). A 5% trichloroacetic acid solution was added according to the rate of 1:1 when the filtrate was cooled to room temperature. Then, the mixture was vortexed for 1 min and then centrifuged at 12000 rpm for 10 min in a centrifuge at 4 °C. The supernatant solution was filtered through a 0.45 μm cellulose syringe filter before injection into the HPLC system. Then the solution was filtered through a 0.45 μm cellulose syringe filter before injection into the HPLC system. The tests were carried out in duplicate.

Table 1. Selected Retention Time Domains Examined for Amino Acids To Yield Three-Dimensional Data for the Second-Order Calibration Method

time segment	analyte	retention time (min)	time region (min) (scan number)
1	Asp	1.85 (292)	1.40−2.28 (220−360)
2	Glu	4.18 (662)	3.47−4.73 (600−750)
3	Asn	6.29 (998)	5.99−6.46 (950−1024)
4	Ser	6.55 (1028)	6.46−6.78 (1024−1075)
5	Gln	7.16 (1135)	6.94−7.57 (1100−1200)
	His	7.38 (1171)	
6	Gly	7.72 (1224)	7.57−8.19 (1200−1300)
	Thr	7.80 (1238)	
7	Arg	8.64 (1371)	8.51−8.86 (1350−1405)
8	Ala	8.97 (1423)	8.82−9.14 (1400−1450)
9	GABA	9.21 (1462)	9.11−9.36 (1445−1485)
10	Thea	9.45 (1500)	9.33−9.64 (1480−1530)
11	Tyr	10.13 (1507)	9.96−10.33 (1580−1640)
12	Val	11.74 (1863)	11.59−11.91 (1840−1890)
13	Met	12.0 (1906)	11.88−12.23 (1885−1940)
14	Trp	12.84 (2038)	12.73−13.03 (2020−2068)
15	Phe	13.12 (2082)	12.98−13.42 (2060−2130)
	Ile	13.23 (2100)	
16	Leu	13.84 (2197)	13.67−14.05 (2170−2230)
17	Lys	14.18 (2251)	14.01−14.36 (2235−2280)

Data Analysis. All calculations were carried out on a microcomputer under the Windows XP operating system. HPLC-FLD data were exported in Microsoft Excel file format using ChemStation software version B.03.01. A background correction program was included in MCRC software (version 1.0)[26] kindly provided by Jalali-Heravi. Chromatography alignment for retention time shift correction was based on Skov's Matlab[27,28] code and in-house-made code. APTLD codes belonging to Wu were implemented, and it was also incorporated into the useful Matlab graphical interface MVC2.[29] The APTLD theory is well documented,[25] so it is not described here.

RESULTS AND DISCUSSION

Chromatography Analysis. In this experiment, a simple gradient elution method is performed as described under HPLC Analysis, and the chromatogram profiles recorded at excitation wavelength (Ex) 230 nm and emission wavelength (Em) 455 nm are shown in Figure 1. As can be seen, HPLC separation of the mixture of 20 standard amino acids is achieved within only 15 min, but peaks show resolutions higher than 1.5 for most of the analytes. The peaks identified in tea infusions are assigned on the basis of comparison of peak retention times listed in Table 1.

In actual tea samples, the coelution problem is apparently due to the complicated matrix, which makes obtaining clear chromatograms difficult for the direct derivatization of tea infusion with OPA. Figure 2 shows the chromatogram profiles of various teas without any pretreatment. Profile a is the mixture of 20 standard amino acids for comparison, and profiles b−f represent two green teas, oolong tea, black tea, and pu-er tea, respectively.

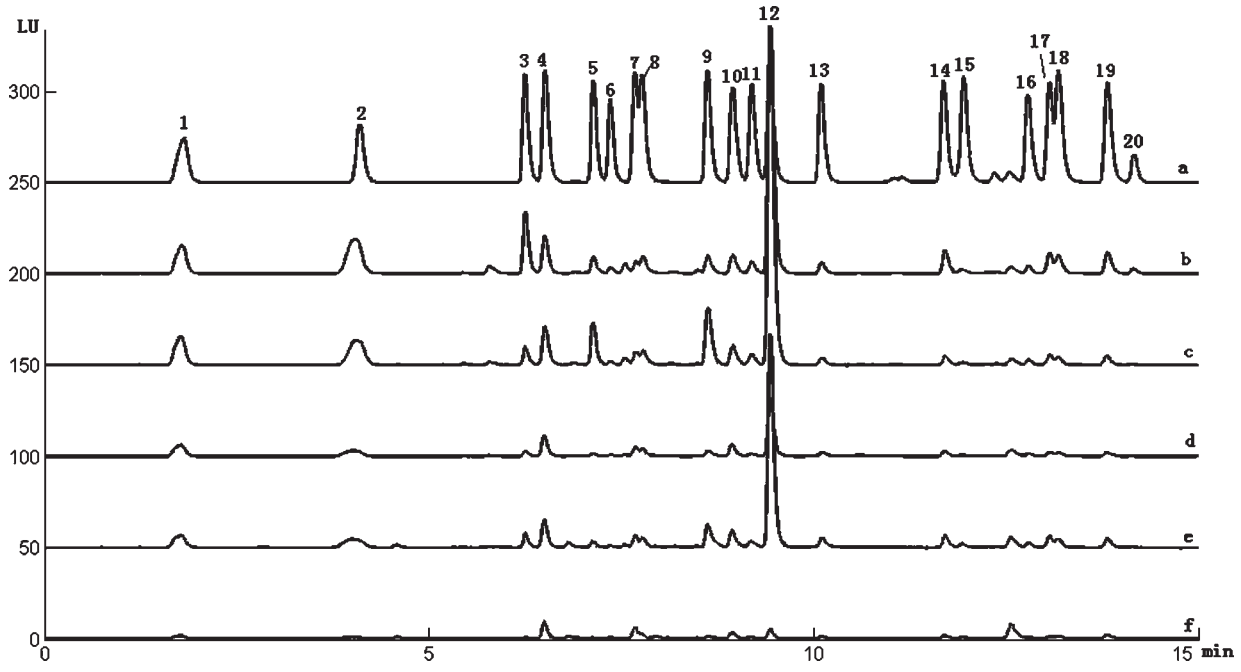

Figure 2. Chromatogram profiles of various teas. a−f represent standard solution, two green teas, oolong tea, black tea, and pu-er tea, respectively.

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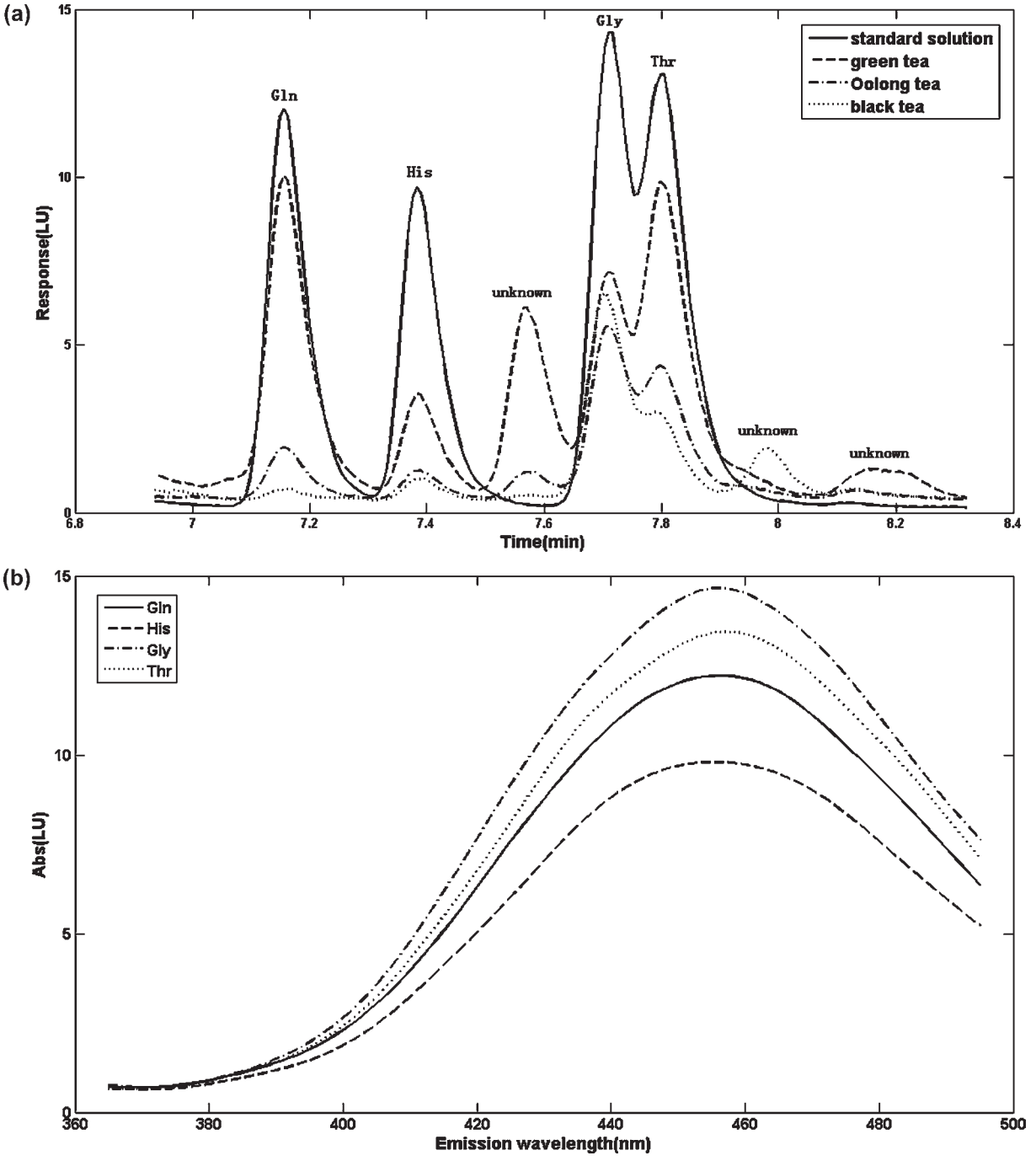

Figure 3. (a) Chromatograms at Ex 230 nm and Em 455 nm for standard solution with a concentration of 50 pmol/μL (solid line), green tea (dashed line), oolong tea (dash-dot line), and black tea (dotted line). (b) Emission spectra of Gln (solid line), His (dashed line), Gly (dash-dot line), and Thr (dotted line) in a corresponding concentration of 50 pmol/μL, respectively.

Several peaks identified as not being amino acid by the standards could not be separated effectively. In fact, similar phenomena have been found in previous studies.[16−18,30] Wang's paper indicated that the phenolic compounds coeluted with the targets might affect the separation and determination of free amino acids.[16] To avoid the interference of sample matrix, complicated sample cleanups[16] or careful optimizations of chromatographic separation conditions[17,18] have been commonly used.

Alternatively, trilinear decomposition algorithms have persistently attracted scientists' interest[31−34] due to their "second-order advantage", which allows for accurate quantification of the analytes of interest even in the presence of uncalibrated interference. This makes it possible to apply "mathematical separation" strategy in complicated tea infusions. In this particular work, a second-order calibration method based on the APTLD algorithm was developed to tackle the problem above.

Calibration and Validation Samples. The calibration curves are respectively obtained in their corresponding time domain with good relationship ($R^2 \geq 0.9999$). To validate the fitted calibration curves, the validation samples were run by APTLD

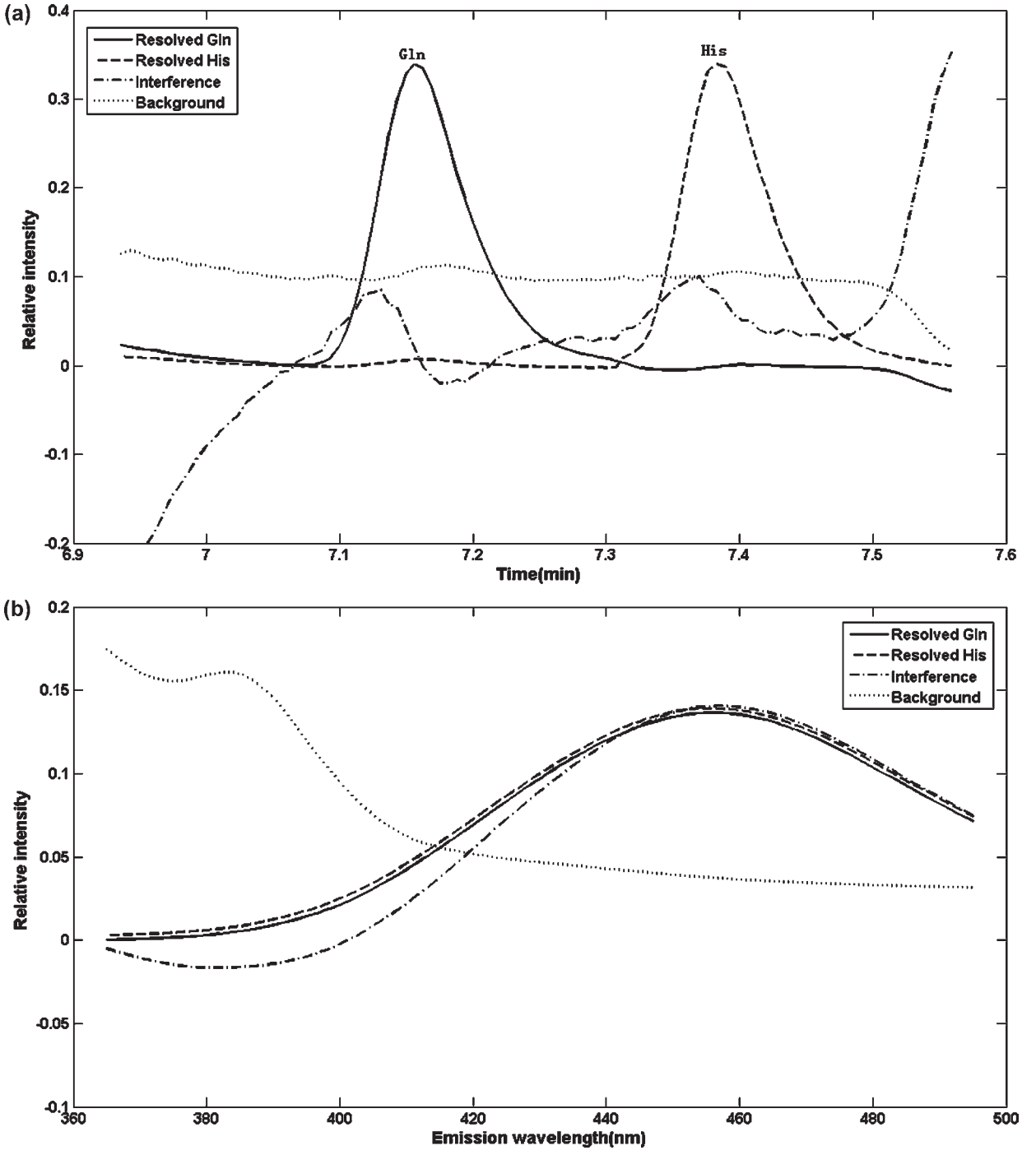

Figure 4. (a) Chromatographic profiles, normalized to unit length, obtained from APTLD when the number of factors was chosen as 4 for the green tea samples and actual elution profiles of Gln (solid line) and His (dashed line). (b) Spectral profiles, normalized to unit length, obtained from APTLD when the number of factors was chosen as 4 for the green tea samples and actual spectral profiles of Gln (solid line) and His (dashed line).

algorithm, and average accuracies ranging from 94.6 to 106.8% are predicted. The results suggested that the APTLD algorithm is suitable for the correct determination of the amino acid content in validation samples. However, the power of "second-order advantage" to overcome the ubiquitous problem of the potential presence of interfering components in tea infusion samples is not completely demonstrated. Therefore, the specific implementation as well as the study of natural matrix samples will be discussed in detail in the following.

Quantitative Analysis of Amino Acids in Tea Infusions. At first, to decrease the complexity, a time segment strategy is necessary, which is the best way of analyzing HPLC multi-wavelength data. When the separation was basically achieved between peaks, analytes were respectively determined in their own elution time domain; otherwise, if peaks overlapped, a common time domain was assigned together. The final result of time segment divisions and the retention time of peaks are listed in Table 1. After the time segments had been analyzed one

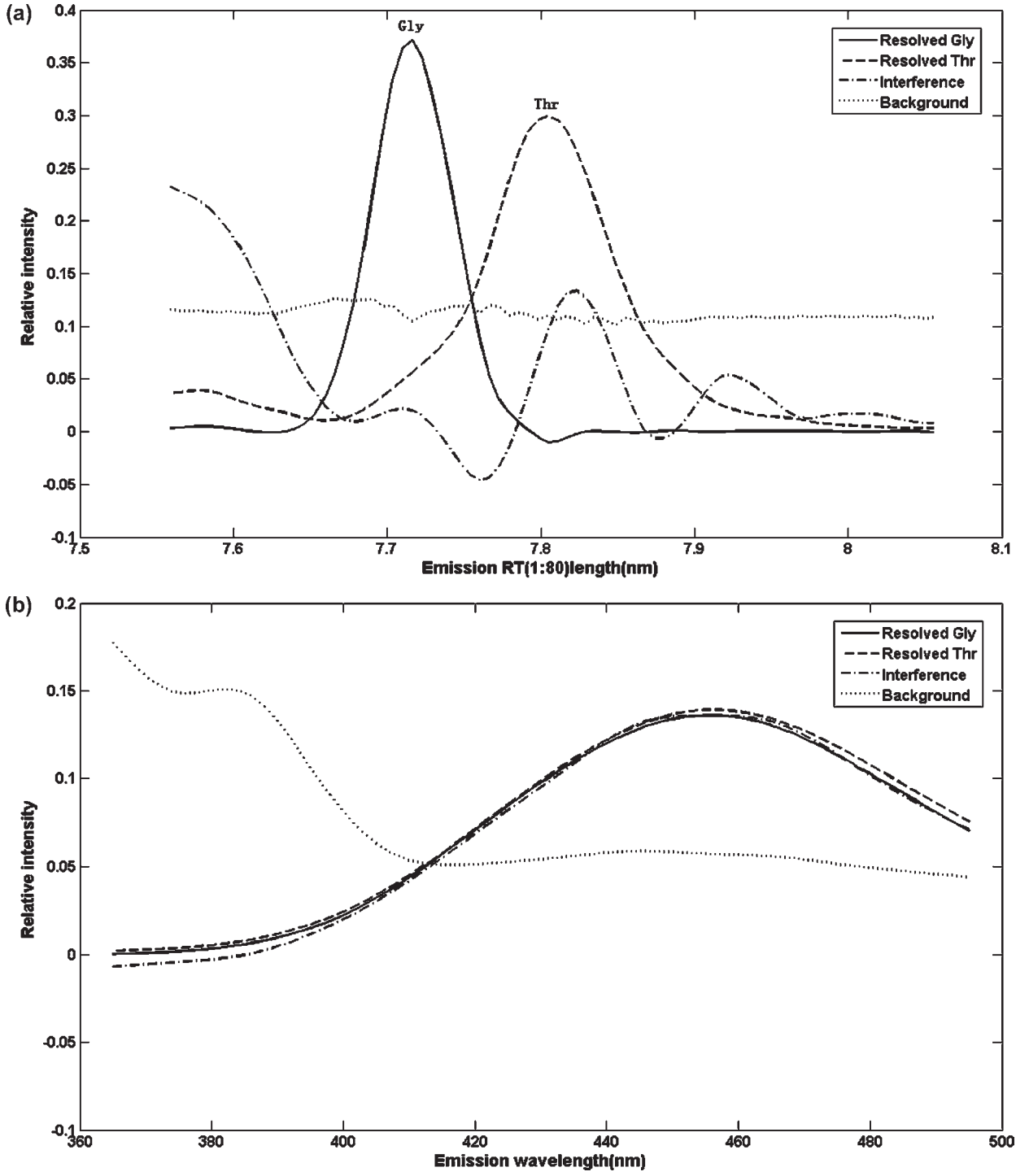

Figure 5. (a) Chromatographic profiles, normalized to unit length, obtained from APTLD when the number of factors was chosen as 4 for the green tea samples and actual elution profiles of Gly (solid line) and Thr (dashed line). (b) Spectral profiles, normalized to unit length, obtained from APTLD when the number of factors was chosen as 4 for the green tea samples and actual spectral profiles of Gly (solid line) and Thr (dashed line).

by one, the final simultaneous determination of free amino acid content in tea infusions was achieved.

For the sake of simplicity, the chromatographic runs monitored between 6.8 and 8.2 min, one of the most complicated segments, was singled out as a representative to demonstrate our work. As can be seen from the chromatography profiles of standard solution (solid line) in Figure 3a, the separation of His and Gly is basically achieved, the Gln and His peaks overlapped slightly, and the Gly and Thr peaks overlapped seriously; that is, the selected raw data are approximately in two different time ranges. For Gln and His, the response data are taken over an elution time range of 6.8−7.6 min, whereas those for Gly and Thr were taken over 7.6−8.1 min.

The emission fluorescence wavelength ranges were all 365−495 nm. The spectral profiles of the four amino acids with concentrations of 50 pmol/uL are shown in Figure 3b. It can be

Table 2. Contents and Recoveries of 20 Free Amino Acids in Teas Predicted by APTLD-Based Method

		green tea 1		green tea 2		oolong tea		black tea		pu-er tea	
no.	AA	contents (mg/kg)	recoveries (%)	contents (mg/kg)	recoveries (%)	contents (mg/kg)	recoveries (%)	contents (mg/kg)	recoveries (%)	contents (mg/kg)	recoveries (%)
1	Asp	2074.9	99.3	2038.5	105.4	665.3	108.1	809.2	104.7	76.4	99.2
2	Glu	3958.9	106.5	2854.4	110.7	611.2	100.4	1225.8	110.7	–	–
3	Asn	2747.9	98.5	624.1	104.2	158.1	107.3	546.9	102.1	39.8	113.6
4	Ser	636.8	96.9	626.6	107.2	179.7	95.5	353.5	110.5	–	86.1
5	Gln	976.3	108.3	1742.8	102.6	120.1	108.4	160.5	107.5	41.2	113.4
6	His	246.1	86.6	130.7	77.1	20.0	90.3	42.9	–	–	–
7	Gly	43.9	107.6	35.1	106.5	16.5	103.2	11.4	111.1	–	–
8	Thr	415.8	97.7	309.0	100.8	102.4	89.6	161.6	101.6	–	–
9	Arg	614.0	77.4	2096.4	83.8	130.7	78.9	774.1	86.5	46.5	85.3
10	Ala	324.2	98.5	316.1	105.0	169.6	111.3	281.3	107.8	25.1	94.6
11	Tyr	399.1	107.4	242.1	96.3	78.8	92.3	304.5	107.1	–	–
12	GABA	320.8	102.7	290.0	111.2	58.3	105.8	170.6	95.0	26.2	104.4
13	Thea	11572.4	107.7	16325.9	109.6	2179.7	111.4	9380.4	108.2	259.4	104.2
14	Val	632.8	97.0	231.3	107.4	86.4	109.9	314.8	108.8	37.8	108.4
15	Met	11.5	–	8.9	–	–	–	–	–	–	–
16	Trp	549.2	99.7	313.4	109.7	115.0	94	345.0	110.9	28.3	120.6
17	Phe	827.6	103.1	393.4	105.2	193.9	110.7	451.4	105.2	23.5	106.5
18	Ile	471.2	109.8	145.8	110.6	76.7	105.7	233.0	108.6	13.3	101.4
19	Leu	670.3	93.5	240.0	102.0	43.9	107.5	226.8	111.4	25.9	100.2
20	Lys	649.0	83.5	253.2	85.8	44.9	79.5	148.5	87.5	41.1	86.4
total (%)		3.77	–	3.32	–	0.52	–	1.67	–	0.07	–

observed that all of the maximum response wavelengths of these analytes are λ_{max} = 455 nm and their spectra are almost parallel and seriously overlapped in the whole spectral range (365−
495 nm). Noticeably, besides the four amino acids shown in Figure 3, the others also have similar and overlapped spectral profiles. This is because most amino acids and the derivatization reagent (OPA) are nonfluorescent themselves; the fluorescence signals observed are from their derivatives, phenylthiocarbamyl (PTC). The approximate structures result in the nice difference in the spectral domain (Figure 3b). Therefore, it is crucial to divide the time domains to avoid interference with one another. In this particular example, two time subsections discussed above are partitioned. Accordingly, for each tea sample, three-way data generated from HPLC-FLD are distributed in three tensors with dimensions 126 × 27 × 11 (for Gln and His) and 95 × 27 × 11 (for Gly and Thr), respectively. The first dimension refers to the chromatographic mode (number of scans; the time domain examined for each analyte is detailed in the last column of Table 1), the second one to the spectral mode (number of wavelengths), and the third to the sample mode (number of calibration samples and prediction samples).

The samples of tea infusions are prepared as described under Sample Preparation. The whole chromatography profiles are arranged in Figure 2. For better resolution, the time window between 6.8 and 8.2 min was enlarged in a new window (Figure 3). It was observed that analytes responded in the selected regions with almost no interference from other components in calibration samples (solid line). Nevertheless, it was obvious that the background and coeluted signals are clear along with various teas, which can impose complexity in chromatographic signals and alter the results of the quantitative process (e.g., recovered spectra of analytes and systematic error in the estimated concentrations in real samples); its proper resolution is of great importance. As one factor model approach, APTLD models the baseline and interferences as individual chemical parts; thereby, additional background elimination is not indispensable in this paper. However, for better evaluation of the results, proper baseline correction[26] was recommended when differences across the samples were pronounced. Moreover, to satisfy the property of trilinearity second-order calibration using APTLD needs, necessary mathematical alignment should be carefully made in each time segment when retention times changed. In this paper, the alignment program was based on Skov's code,[27,28] which can be freely downloaded from the Website (http://www.models.kvl.dk/algorithms).

Although the APTLD algorithm is insensitive to the component number, it can perform well with more acceptable calibration and resolution and give better results when the most appropriate factors have been adopted. Referring to the suggestion of the core consistency diagnostic (CORCONDIA),[33] two four-component models are built for Gln and His in a 126 × 27 × 11 three-way data array and for Gly and Thr in a 95 × 27 × 11 three-way data array, respectively. Of these, two factors are modeled for analytes, one corresponding to the interferences and the remaining one being mainly background.

The APTLD-resolved chromatographic profiles as well as spectral profiles of the analytes in green tea infusion are shown in Figures 4 and 5. The loadings associated with the chromatographic mode are shown in Figures 4a and 5a, and the loadings associated with the spectral mode are shown in Figures 4b and 5b. As can be seen, although the peaks overlapped seriously and fluorescence spectra were almost identical, APTLD still successfully deconvoluted the target analytes (solid line and dash line)

Table 3. Analytical Figures of Merit for Determination of Amino Acids by APTLD Modeling

	figures of merit for tea infusions			
analyte	SEN	SEL	LOD (pmol/μL)	LOQ (pmol/μL)
Asp	5.9	0.7	0.43	1.44
Glu	2.1	0.74	0.58	1.93
Asn	2.7	0.57	1.50	5.00
Ser	3.3	0.64	1.20	4.00
Gln	4.5	1.02	1.12	3.73
His	3.8	0.95	0.91	3.03
Gly	4.3	0.84	1.21	4.03
Thr	3.8	0.89	0.81	2.73
Arg	2.1	1.02	0.46	1.53
Ala	3.2	1.02	0.95	3.16
GABA	3.4	1.24	0.93	3.13
Thea	5.4	1.56	1.78	5.93
Tyr	2.0	0.93	1.03	3.43
Val	2.6	0.98	0.76	2.54
Met	3.1	1.31	0.82	2.75
Trp	4.3	1.34	0.57	1.92
Phe	4.9	0.74	0.74	2.47
Ile	3.6	1.43	0.67	2.24
Leu	2.2	0.57	0.45	1.51
Lys	2.5	0.69	1.02	3.41

free from the interferences (dash-dot lines) and background (dotted lines) with the aid of the strategies discussed above. Noticeably, negative parts and irregular interference curves are observed in Figures 4 and 5, which may be caused by incomplete deconvolution of the background components, other interferences, predicted errors, etc.

Besides the four amino acids discussed above, the other amino acid contents are obtained in the same manner; the process is not described in detail any further. As for the time domains with a single amino acid, the APTLD-based model still helps to achieve more accurate results due to the elimination of potential background components or other interferences.

The prediction results for all amino acids based on the APTLD algorithm are summarized in Table 2, which shows that Thea is the most abundant amino acid and accounts for >50% of the total amino acids in all teas. Green tea has a much higher Thea content, with >1% in dry teas; black tea has the second highest amount, and pu-er tea has the least. Furthermore, similar relationships of total amounts of free amino acids are also present. The results are in general agreement with those of previous studies.[16,17] The reason for this phenomenon is not the emphasis herein; the reader can find professional literature about tea. To further validate the performance of the proposed method, experiments for recoveries were designed. Additional amounts of 80, 100, and 200% of free amino acids are added into tea powers according to the determination results in Table 2 before sample preparation, and the following steps are run as described under Sample Preparation. The average recoveries of the three levels above gained from the APTLD algorithm are also listed in Table 2. Some amino acids are not added because they are not detected (−) in teas (for example, Gly and Thr in pu-er tea). The recoveries range from 80 to 120%, highlighting that the second-order calibration method based on the APTLD algorithm is capable of reliably quantitatively analyzing amino acids in complex tea infusion samples.

Statistical Validation and Figures of Merit. To evaluate the performance of the developed method, the analytical figures of merit including sensitivity (SEN), selectivity (SEL), limit of detection (LOD), and limit of quantification (LOQ) have been calculated. For the sake of simplicity, the maximum values of the parameters are listed in Table 3. Obviously, most LODs are approximately 1.0 pmol/μL and LOQs are 3.0 pmol/μL, which are far lower than traditional method LODs and LOQs of 3.0 and 10.0 pmol/μL. Although Thea has the highest LOD and LOQ, it is nonessential for the accurate quantification because of its high content in tea.

In summary, a rapid and effective method to simultaneously determine the contents of free amino acids in tea has been successfully developed with the aid of APTLD method. Nowadays, automatic amino acid analysis is widely used to profit from its excellent stabilization. Unfortunately, it requires 90−150 min, and it cannot achieve such complicated separation due to the unexpected interferences. Alternatively, the APTLD-based method can accomplish it within only 20 min, and the calculations are automatic. Being provided with the "second-order advantage", APTLD could predict the accurate concentrations together with reasonable resolution of chromatographic and spectral profiles for the analytes of interest even in complex systems. The results obtained indicate that it is an attractive alternative strategy in routine determination of free amino acids in tea. Furthermore, it is also anticipated to expand the application or improve existing methods for complicated work and significantly decreases both experimental time and complexity.

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ABBREVIATIONS USED

APTLD, alternating penalty trilinear decomposition; Ex, excitation wavelength; Em, emission wavelength; PARAFAC, parallel factor analysis; HPLC-FLD, high-performance liquid chromatography with fluorescence detection; RP-HPLC, reversed-phase high-performance liquid chromatography; Thea, theanine; GABA, γ-aminobutyric acid; OPA, *O*-phthalaldehyde; SEN, sensitivity; SEL, selectivity; LOD, limit of detection; LOQ, limit of quantification.

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