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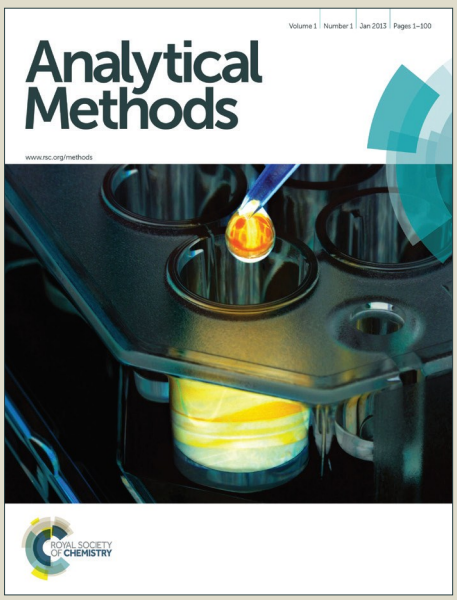

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Highly efficient and sensitive screening of ractopamine in foodstuffs by HPLC-FLD using fluorescent labeling and ultrasonic-assisted dispersive liquid-liquid microextraction

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Abstract

A simple, low-cost, rapid, sensitive and specific analytical method for ractopamine (RAC) screening in foodstuffs based on fluorescent labeling coupled with ultrasonic-assisted dispersive liquid-liquid microextraction (UA-DLLME) was established by high-performance liquid chromatography (HPLC) with fluorescence detection (FLD). In this study, 2-(11H-benzo[*a*]carbazol-11-yl) ethyl chloroformate (BCEC-Cl) was first employed as the labeling reagent for RAC, and the labeled sample was directly submitted to UA-DLLME. In order to obtain the optimal experimental conditions, several important parameters including the kinds of extraction and dispersive solvent, pH of the solution, salt effect and the volume of sample solution affecting the UA-DLLME were optimized systematically. Moreover, the main parameters including ultrasonic time (T), extraction solvent volume (EV) and disperser solvent volume (DV) were further optimized by response surface methodology (RSM) based on a Box-Behnken design (BBD). Under the optimal conditions, the quantitative linear range of targeted analyte was 2 – 100 ng mL^{-1}, and the correlation coefficient was 0.9995. The low limit of detection (LOD) and limit of quantification (LOQ) for RAC were 0.4 ng mL^{-1} and 1.3 ng mL^{-1}, respectively. The developed method combing the merit of fluorescent labeling and UA-DLLME facilitated the high-sensitivity and high-throughput sample screening. The novel method was successfully applied to the trace RAC screening in foodstuffs with excellent applicability and good reproducibility. This method was proven to be inexpensive, sensitive, efficient, accurate and reliable for trace RAC screening.

1. Introduction

Ractopamine (RAC) is a typical β-adrenergic agonist, which can be used for the treatment of respiratory diseases in clinical medicine.[1,2] RAC also has the function to increase the percentage of lean meat and improve feed conversion ratio.[3-5] It is often illicitly abused as growth promoters in livestock by the promotion of repartitioning of fat into muscles and as doping drugs to enhance the performance of athletes.[6] Although the usage of RAC as animal growth accelerant is strictly banned in most countries and areas (over 150) around the world, such as China, Japan and Europe,[7,8] many cases of acute food poisoning still happened in the presence of such drug residues in foods. Thus, development of a low-cost, rapid, sensitive and specific analytical method for RAC screening in foodstuffs is an urgent task.

Until recently, many analytical methods including enzyme immunoassay (EIA),[9] enzyme-linked immunosorbent analysis (ELISA),[10] capillary electrophoresis,[1] surface plasmon resonance-based biosensor inhibition immunoassay,[11] high-performance liquid chromatography (HPLC),[12] liquid chromatography-mass spectrometry (LC-MS),[13-17] and gas chromatography-mass spectrometry (GC-MS)[8] have been developed for RAC detection. Although each of these methods possesses their own features and gave the different insights for RAC analysis in animal tissues, urine and feed, they show some disadvantages during the practical application. For example, EIA and ELISA were developed in the past and are still being used in many countries for the control of RAC. These techniques often suffer from the limited cross-reactivity of antibodies, which is not suitable for confirmation of RAC. GC-MS has been widely used for the identification and confirmation of RAC during the last few years. However, GC-MS requires a long time for derivation because of their high polarity and low volatility, which is time-consuming, tedious and expensive. As an alternative of GC-MS, HPLC- MS is a more important and effective method in the analysis of RAC. But while LC-MS adopts in routine use for quantitative analysis, the expensive isotope internal standard is necessary and the equipment of HPLC-MS is also expensive, not easily available in common analytical laboratories. With the advantages of high selectivity, sensitivity and applicability, HPLC-FLD has been widely used in the determination of trace targeted compounds,[18-20] which is much more preferable to the analysis of RAC. But RAC possesses none fluorescence, the direct fluorescence detection is not suitable. Thus, fluorescent labeling of RAC before analysis is feasible, which endows RAC with fluorescent property and

significantly improves detection sensitivity. Furthermore, in the reported methods above, the sample pretreatment processes for RAC extraction are mainly based on SPE,[1] which requires a long extraction time, large sample volume, and large organic solvent consumption. Thus, there is a need to develop a green analytical methodology that is capable of possessing high enrichment efficiency, less extraction time and small sample volume. Recently, ultrasonic-assisted dispersive liquid-liquid microextraction (UA-DLLME) as an emerging technique has the advantages of simple and rapid operations, significant timesaving, low consumption of chemical reagents, good recovery and high enrichment factor.[21-24] In the presented study, we first established a rapid, reliable, sensitive and cost-effective method based on fluorescent labeling followed by UA-DLLME for RAC screening by HPLC-FLD. The chemical construct of RAC was not suitable for UA-DLLME because of its strong polarity (possessing hydroxyl group, phenolic hydroxyl group and amine group). In this study, 2-(11H- benzo[*a*]carbazol-11-yl) ethyl chloroformate (BCEC-Cl) as an excellent fluorescent reagent was employed to label RAC, ensuring highly sensitive detection. Furthermore, after fluorescent labeling, the polarity of RAC was decreased significantly, vastly facilitating the following UA-DLLME. The experimental variables affecting UA-DLLME were systematically investigated and optimized by response surface methodology (RSM) based on a Box-Behnken design (BBD).[25,26] The fluorescent labeling and UA-DLLME were achieved in 10 min and 2.4 min, respectively, and the followed HPLC separation of RAC was obtained in 8 min. Under the optimized conditions, an ultralow detection limit (0.4 ng mL^{-1}) and quantification limit (1.3 ng mL^{-1}) were achieved, indicating the higher detection sensitivity than the reported methods.[1,8,15,27-29] The proposed method was also validated and successfully applied to the quantitative analysis of RAC in real samples including pork, chicken and fish. Overall, the developed method based on fluorescent labeling followed by UA-DLLME for the determination of RAC by HPLC-FLD was proven to be rapid, efficient, sensitive and accurate for RAC screening in meat products.

2. Materials and methods

2.1 Chemicals and reagents

2-(11H-benzo[*a*]carbazol-11-yl) ethyl carbonochloridate (BCEC-Cl) was synthesized in our previous study.[30] Ractopamine (RAC) was purchased from Sigma (St. Louis, MO, USA). Double distilled water was produced by a Milli-Q system (Millipore, Bedford, MA, USA). Acetonitrile

(HPLC grade), methanol, dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), carbon tetrachloride (CCl_4) and acetone were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Other chemicals were analytical grade from Jining Chemical Reagent (Jining, Shandong Province, China). RAC stock solution (1×10^{-3} mol L^{-1}) was prepared in methanol. The fluorescent labeling reagent solution (5×10^{-3} mol L^{-1}) was prepared by dissolving 16.15 mg of BCEC-Cl in 10 mL acetonitrile.

2.2 Instrumentation and analytical conditions

An Agilent 1100 HPLC system was used for HPLC analysis, which was coupled on-line to a fluorescence detector. A Hypersil C18 (4.6 mm × 200 mm, 5 μm) column that was purchased from Dalian Elite Analytical Instruments Co., Ltd (Dalian, China) was used for the separation of targeted analyte. The column temperature was kept at 30 °C, and the injection volume was 10 μL. The mobile phase was composed of water containing 5 % acetonitrile (A) and acetonitrile (B), and the flow rate was maintained at 1 mL min^{-1}. The gradient was programmed to linearly increase the amount of mobile phase B as follows: 0~3 min (60 – 81 %), 3~5 min (81 – 85 %), 5~7 min (85 – 100 %) and 7~8 min (100 – 100 %). The excitation and emission wavelengths were set at 279 nm and 380 nm, respectively.

2.3 Sample preparation

All foodstuffs including pork, chicken and fish were purchased from a local supermarket (Qufu, Shandong Province, China). The sample preparation was carried out by referring several recent studies with minor improvement.[13,27] 1 g of samples were accurately weighed and extracted twice with 4 mL of acetonitrile-water (80:20, *v/v*) in an ultrasonic cleaner (5 min each extraction), and centrifuged at 5000 rpm for 5 min. Subsequently, the collected supernatants were dried under nitrogen, re-dissolved with 1 mL water and then filtered through 0.22 μm nylon filter for use.

2.4 Fluorescent labeling of RAC and UA-DLLME

The derivatization scheme of RAC with BCEC-Cl is shown in **Fig. 1**. To a calibrated 10 mL conical test tube, 4 mL sodium borate buffer (pH = 9.0) containing 40 μL mixed standard solution (or real sample extracting solutions) and 60 μL fluorescent reagent solutions were added in order. The tube was shaken for 10 s and then allowed to stand for 10 min at 50 °C. After fluorescent labeling, the mixture was immediately extracted by UA-DLLME. Prior to UA-DLLME procedure, 10 μL NaCl (1 %, *w/v*) was placed in the glass tube. Then 1345 μL of methanol (as disperser

solvent) and 136 μL $CHCl_3$ (as extraction solvent) were mixed, and rapidly injected into the sample solutions by using the 2 mL glass syringe. And then the tube was immersed in an ultrasonic water bath for 2.4 min. Subsequently, the mixture was centrifuged at 5000 rpm for 3 min. The upper aqueous phase was removed, and the sedimentary phase was evaporated to dryness by a gentle nitrogen stream. Then, the remnant was re-dissolved by 0.5 mL methanol and filtered with a 0.45 μm filter for further analysis.

2.5 Method validation

The established analytical method was extensively validated according to the USP guideline and several reported studies.[31,32] The HPLC method was validated by the calibration curves, limit of detection (LOD), limit of quantification (LOQ), repeatability, precision, accuracy, robustness and specificity, respectively. Calibration curve of RAC was established by plotting peak areas of RAC (y) versus their corresponding concentrations (x) in the range of 2 – 100 ng mL^{-1}. LOD and LOQ were measured at the signal-to-noise of 3:1 (S/N = 3:1) and at signal-to-noise of 10:1 (S/N = 10:1), respectively. The repeatability was validated by injecting 10 μL RAC standard sample into the chromatograph six times under identical conditions to measure the relative standard deviations (RSD) for peak areas and retention times. The precision was analyzed by intra- and inter-day tests containing six replicates over 3 days. The accuracy was determined by spiking with three different levels of the standard (1 μg/kg, 5 μg/kg and 10 μg/kg) into all real samples and the recoveries were analyzed. The robustness was investigated by making small changes in separation conditions and monitored the changes in separation and detection. The specificity was validated by injecting different solutions including (a) an injection solvent (mobile phase B), (b) solutions of standards, (c) extracted blank sample solutions and (d) extracted blank sample solutions fortified with internal standards.

3. Results and discussion

3.1 HPLC separation

In order to obtain an ideal chromatographic separation, different mobile phases (methanol and acetonitrile) and analytical columns including Hypersil C18 (4.6 mm × 200 mm, 5 μm) column, Eclipse XDB-C8 (4.6 mm × 150 mm, 5 μm) column, Hypersil BDS C8 (4.6 mm × 200 mm, 5 μm) column, Spherisorb C18 (4.6 mm × 200 mm, 5 μm) and Hypersil BDS-C18 column (4.6 mm × 200 mm, 5 μm) were evaluated. Based on the data analysis, a Hypersil C18 (4.6 mm ×

200 mm, 5 μm) was selected for the gradient elution. A series of experiments showed that acetonitrile/water offered a more rapid elution program of RAC derivative than methanol/water. The optimum mobile phase composition was 5 % acetonitrile (A) and acetonitrile (B). Moreover, the flow rate was maintained at 1 mL min^{-1} and the column temperature was constant at 30 °C. Under the proposed conditions, the typical chromatogram of RAC standard solution was shown in **Fig. 2A**, and RAC derivative could be separated within 8 min.

3.2 Optimization of UA-DLLME conditions

In order to obtain high extraction efficiency, several important parameters affecting the UA-DLLME, including the kinds of extraction and dispersive solvent, pH of the solution, salt effect and the volume of sample solution, were optimized step by step. The extraction time (T), the volume of extraction (EV) and dispersive solvent (DV) were further optimized by a three-level, three-variable BBD from RSM.

Extraction solvent can significantly affect extraction efficiency in UA-DLLME. The essential requirements for an adequate extraction solvent include low solubility in water, high affinity to the targeted analyte and larger density than water for achieving an ease collection of the sedimented phase. Taking into consideration of the mentioned factors above, mainly chlorinated solvents (e.g. CH_2Cl_2, $CHCl_3$ and CCl_4) were focused, which were examined systematically (**Fig. 3A**). The results indicated each of the evaluated solvents could obtain a certain effect, but the $CHCl_3$ give a little better extraction effect than others. This may be due to that the fluorescent labeling derivative of RAC has the functional groups of hydroxyl and tertiary amine and possesses certain polarity (**Fig. 1**), and RAC derivative is more inclined to be extracted by $CHCl_3$ because the polarity of $CHCl_3$ is little larger than that of CH_2Cl_2 and CCl_4. Thus, $CHCl_3$ was chosen as the extractant in UA-DLLME.

The miscibility of dispersive solvent with both organic extractant and aqueous phase is the main criterion for the selection of dispersive solvent in UA-DLLME. Several organic solvents including acetonitrile, acetone, methanol and ethanol were tried, which are often used as the dispersive solvent in UA-DLLME (**Fig. 3B**). The results revealed that acetonitrile gave higher fluorescence intensity compared to the others. Thus, acetonitrile was selected as the dispersant in UA-DLLME.

The pH of the sample solution can significantly affect the extraction efficiency especially

when the targeted compound with alkaline or acidity. In this study, the effect of solution pH (ranging from 4 to 10) was investigated systematically (**Fig. 3C**), and results in **Fig. 3C** indicate the varying pH has no obvious effect on response values. It is mainly because of the phenolic hydroxyl group and amine group of RAC have been derived by the fluorescent reagent. Hence, the neutral pH was selected in UA-DLLME.

The increase of the ionic strength can lead to a decrease in the solubility of the analyte in sample solution; thus, extraction efficiency may be enhanced. For investigating the influence of ionic strength on the extraction efficiency of UA-DLLME, various experiments were performed by adding different concentrations of NaCl (0 – 5 %, *w/v*) with other experimental conditions keeping constant (**Fig. 3D**). Result showed the increase of ionic strength will not significantly affect the extraction efficiency; on the contrary, the higher ionic strength was harmful to the UA-DLLME of RAC. Therefore, the NaCl (1 %, *w/v*) was selected in UA-DLLME.

On the basis of current knowledge, the extraction efficiency of UA-DLLME could be improved by increasing the sample volume, because an increasing sample volume could offer a positive-going effect for transferring the targeted compound from the aqueous phase into the extraction phase.[23] In this paper, the effect of sample volume, ranging from 1.5 to 5.5 mL, was investigated. **Fig. 3E** indicates that the peak area changes slightly with the increase of sample volume from 1.5 to 4 mL, but the peak area decreases with the increase of sample volume from 4.5 to 5.5 mL. Finally, the volume of 4 mL was selected in UA-DLLME.

3.3 Further optimization of UA-DLLME by RSM

The main parameters including T, EV and DV were optimized by a three-level, three-variable BBD from RSM. According to BBD design, a total of 17 runs are given in **Table 1**, and the peak area of RAC was selected as the response variable. Based on the experimental data, a regression equation that could predict the optimum point was obtained. The predicted second-order polynomial model was as follows:

$$Y = 9.61 - 0.39\,X_1 + 2.40\,X_2 + 1.80\,X_3 - 1.50\,X_1X_2 + 1.81\,X_1X_3 - 0.68\,X_2X_3 - 2.47\,X_1^2 - 1.19\,X_2^2 - 3.09\,X_3^2$$

Where, Y is the predicted average peak areas; X_1, X_2 and X_3 are the coded values of ultrasonic time (T), extraction solvent volume (EV) and disperser solvent volume (DV), respectively.

Three-dimensional response surface curves are shown in **Fig. 4**. **Fig. 4A** demonstrates the interaction between T and EV on the response value at the fixed value of DV. In **Fig. 4A**, when the T increases from 2 to 6 min, the response value rapidly increases and reaches a maximum value, and then slightly decreases. With the increase of EV from 50 to 150 μL, the response value was increased. **Fig. 4B** shows the remarkable interaction between T and DV. With a given EV, the peak area increased with the increase of DV and reached the highest value around 1250 μL, and then a little decline was observed with its further increases. The tendency of T is basically consistent with **Fig. 4A**. **Fig. 4C** describes the interaction between EV and DV on the response value at the fixed value of T. As **Fig. 4C** described, by improving the amount of DV, the peak area increases and reaches a maximum value, followed by a decline. And the peak area increases with the increasing amount of EV.

The results of the analysis of variance (ANOVA) indicated the fitness was significant at the level of $p < 0.05$. The value of R^2 (expressing the variability in the response) was 0.8315, indicating that the experimental and predicted value reached a satisfactory agreement. The optimal conditions were given by RSM as follows: extraction solvent volume = 136 μL, disperser solvent volume = 1345 μL and sonication time = 2.4 min. Under the proposed conditions, peak area was predicted to be 8.75. The suitability of the optimal UA-DLLME conditions above were also tested by executing six experiments and the average peak area was 9.13, which was very close to the predicted value. The excellent correlation between predicted and measured values verified that the response model was adequate to reflect the expected optimization.

3.4 Method validation

The proposed method was validated by linearity, LOD, LOQ, repeatability, precision, accuracy, robustness and specificity. The linearity of the proposed method was tested with calibration curve using seven calibration points in the concentration range 2 – 100 ng mL^{-1} and the calibration curve had excellent correlation coefficient of 0.9995 (**Table 2**). As can be seen in **Table 2**, quite low LOD (0.4 ng mL^{-1}, S/N = 3:1) and LOQ (1.3 ng mL^{-1}, S/N = 10:1) for RAC derivative with fluorescence detection are obtained, which indicated that the proposed method has favorable analytical sensitivity. To corroborate the repeatability of the proposed method, quantitative RAC derivative was injected six times under the same optimum chromatographic conditions. The satisfactory repeatability for retention times and peak areas were obtained, which demonstrated

that the proposed method was reliable. For further validation of the precision of the proposed method, the comparison including six replicates intra- and inter-day tests was taken up. The intra- and inter-day precision (expressed in terms of RSD %) were 3.7 % and 6.1 %, respectively (**Table 2**). For the accuracy, three different levels of the standard (1 μg/kg, 5 μg/kg and 10 μg/kg) were spiked into real samples and the recoveries were analyzed. And analytical results for the recovery of real samples are listed in **Table 3**. The average recoveries were in the range of 94.0 – 101.2 % and the RSD values were less than 3.4 %. For the robustness, small variations in separation conditions were made to investigate the changes in separation and detection. For instance, the effect of flow rate and column temperature were studied by analyzing samples at 1 ± 0.1 mL min^{-1} and 30 ± 1 °C, respectively. The negligible changes were observed, indicating acceptable robustness. For the specificity, a comparison was evaluated with injecting the following solutions: (a) an injection solvent (mobile phase B), (b) solutions of standards, (c) extracted blank sample solutions and (d) extracted blank sample solutions fortified with internal standard. The absence of interference peaks in the solvent and solutions, indicating no interference from other compounds. Additionally, all of the peaks were well-resolved in the standard mixture chromatograms. Therefore, the method has good specificity. In summary, the validation data demonstrates that the proposed method has good linearity, satisfactory repeatability, acceptable robustness, favorable precision, accuracy and specificity for RAC screening in foodstuffs.

3.5 Comparison with the reported methods

The comparison of the proposed method with reported methods was listed in **Table 4**. As is shown in **Table 4**, analytical method, sample treatment, LOD and LOQ are listed for the comparison. For the sample treatments, compared to SPE[1] and MEPS,[27] UA-DLLME could overcome the disadvantages of SPE and MEPS with the advantages of simple and rapid operation, significant timesaving, low consumption of chemical reagents, good recovery and high enrichment factor. The proposed method also possessed lower LOD and LOQ than the reported methods (**Table 4**),[1,8,15,27-29] indicating higher sensitivity. MS method could provide a desired detection limit, but need the expensive isotope internal standard and equipment, and it is not easily available in common analytical laboratories. Compared with MS, HPLC-FLD with high efficiency and sensitivity was widely used in common laboratory. Furthermore, the fluorescent labeling and the followed UA-DLLME were rapidly achieved in 10 min and 2.4 min, respectively. Overall, the

developed method based on fluorescent labeling followed by UA-DLLME for RAC screening by HPLC-FLD was proven to be rapid, efficient, sensitive and accurate in meat products.

3.6 Application to the analysis of foodstuffs

As an illicit food additive, the usage of RAC is strictly banned in most countries and areas around the world. Therefore, RAC screening in foodstuffs is significant. In this study, the established method was applied for RAC screening in foodstuffs samples including pork, chicken, and fish. Using the methods described herein, RAC has not been detected in all samples (n=6) (**Table 3**). It should be pointed out that the misuse of RAC is efficaciously controlled in local food-producing. And the typical chromatogram of pork sample is shown in **Fig. 2B**.

4. Conclusion

In this paper, a novel HPLC-FLD method coupled with pre-column labeling using BCEC-Cl as a fluorescent reagent for RAC screening was established and validated. Subsequently, UA-DLLME as an emerging technique was employed for sample treatment. In order to obtain the optimal experimental conditions, the important parameters were optimized systematically. Under the optimal conditions, the developed method was successfully applied to real sample determination including pork, chicken and fish. The presented method possessed the advantages including rapidity, low cost, high efficiency and excellent sensitivity. Moreover, this method could be further extended for RAC screening in many other samples.

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Conflict of Interest:

Mei Zhao declares that she has no conflict of interest. Guoliang Li declares that he has no conflict of interest. Nannan Qiu declares that he has no conflict of interest. Guang Chen declares that he has no conflict of interest. Lian Xia declares that she has no conflict of interest. Jinmao You declares that he has no conflict of interest. Yongning Wu declares that he has no conflict of interest.

Ethical Approval:

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent: Not applicable.

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Figure captions

Fig. 1. The derivatization scheme of RAC with BCEC-Cl.

Fig. 2. The representative chromatograms for standard (A) and pork sample (B).

Fig. 3. The optimization of UA-DLLME conditions, including kinds of extraction (A), dispersive solvents (B), pH (C), salt effect (D) and the volumes of sample solution (E).

Fig. 4. The response surface plots for optimization of UA-DLLME conditions, including the peak area affected by varying T and EV (A), the peak area affected by varying T and DV (B), the peak area affected by varying EV and DV (C).

Tables

Table 1 The experimental results of the optimization of UA-DLLME parameters by RSM

Run	Parameters			Area
	T	EV	DV	
1	4(0) [a]	150(+1) [a]	1500(+1) [a]	7.48
2	2(–1)	100(0)	1500(+1)	2.93
3	4(0)	50(–1)	1500(+1)	7.54
4	6(+1)	100(0)	500(–1)	1.56
5	4(0)	100(0)	1000(0)	9.51
6	6(+1)	150(+1)	1000(0)	7.09
7	4(0)	100(0)	1000(0)	9.65
8	2(–1)	100(0)	500(–1)	3.72
9	6(+1)	100(0)	1500(+1)	8.02
10	2(–1)	50(–1)	1000(0)	1.81
11	4(0)	100(0)	1000(0)	9.73
12	4(0)	100(0)	1000(0)	9.49
13	2(–1)	150(+1)	1000(0)	13.1
14	6(+1)	50(–1)	1000(0)	1.81
15	4(0)	50(–1)	500(–1)	1.83
16	4(0)	150(+1)	500(–1)	4.48
17	4(0)	100(0)	1000(0)	9.67

[a] actual level (coded level).

Table 2 Linear regression equation, correlation coefficient (R), LOD, LOQ, repeatability of retention time and peak area, intra- and inter-day precision.

Analyte	Regression equation	LOD ($ng\ mL^{-1}$)	LOQ ($ng\ mL^{-1}$)	Repeatability RSD (%)(n=6)		Intra-day precision (RSD %, n =6)	Inter-day precision (RSD %, n =6)
				Retention time	Peak area		
RAC	y=14.2x–3.51[a] R= 0.9995[b]	0.4	1.3	0.02	0.46	3.7	6.1

[a] y: peak area detected with fluorescence detector; x: the injected amount (pg); 10 μL injection volume.

[b] Regression coefficient.

Table 3 Analytical results for the recovery of real samples (n= 6)

Analyte	Original (μg/kg)	Added (μg/kg)	Found (μg/kg)	Recovery (%)	RSD (%)
Pork	0	1	0.99	99.0	1.7
	0	5	4.90	98.1	2.6
	0	10	10.1	101.2	2.8
Chicken	0	1	0.96	96.0	2.9
	0	5	4.81	96.2	3.4
	0	10	9.92	99.2	3.2
Fish	0	1	0.94	94.0	2.7
	0	5	4.89	97.8	2.9
	0	10	9.90	99.1	2.5

Table 4 Compared with reported methods

Analytical method	Sample treatment	LOD ($ng\ mL^{-1}$)	LOQ ($ng\ mL^{-1}$)	References
CE-ED [a]	SPE	90	310	[1]
HPLC-UV [b]	MEPS[c]	3	10	[27]
LC-MS	–[d]	10	–	[15]
GC-MS	–	3.6	–	[8]
Electrochemical method	–	20	–	[28]
Colorimetric detection	–	–	108.7	[29]

[a] Capillary electrophoresis with electrochemical detection.

[b] High-performance liquid chromatography–ultraviolet detection.

[c] Microextraction by packed sorbent.

[d] not mentioned in the method.

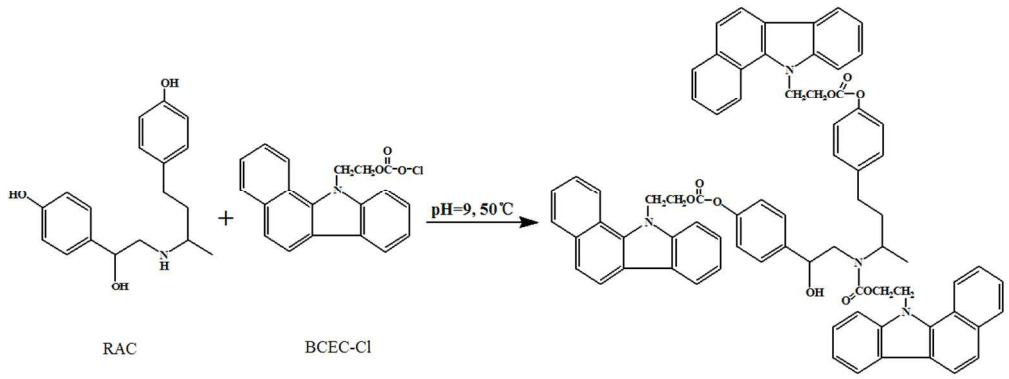

Figure 1
327x122mm (96 x 96 DPI)

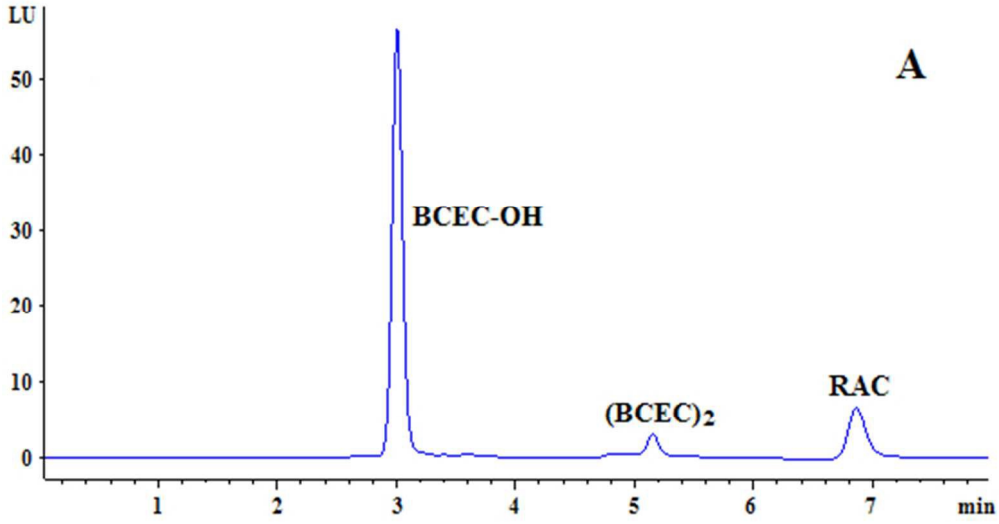

Figure 2A
145x76mm (96 x 96 DPI)

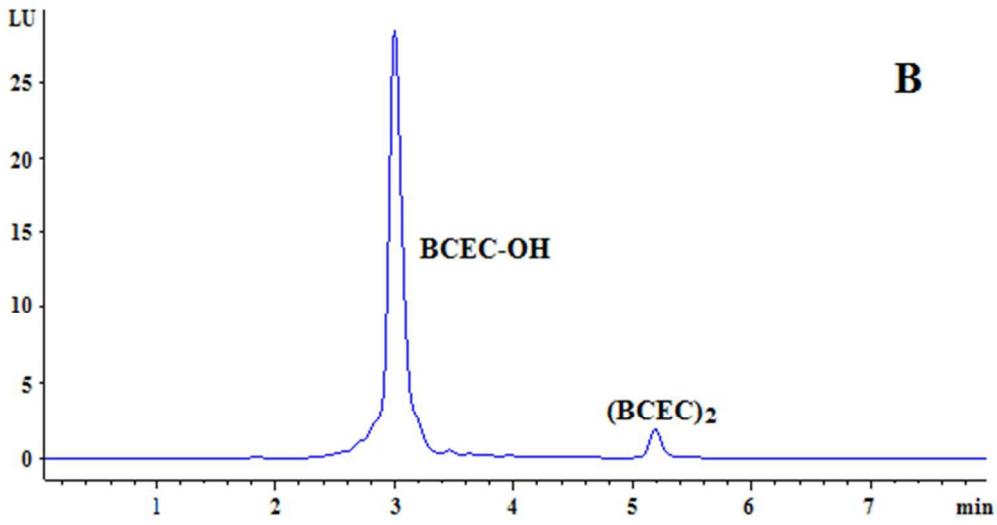

Figure 2B
145x75mm (96 x 96 DPI)

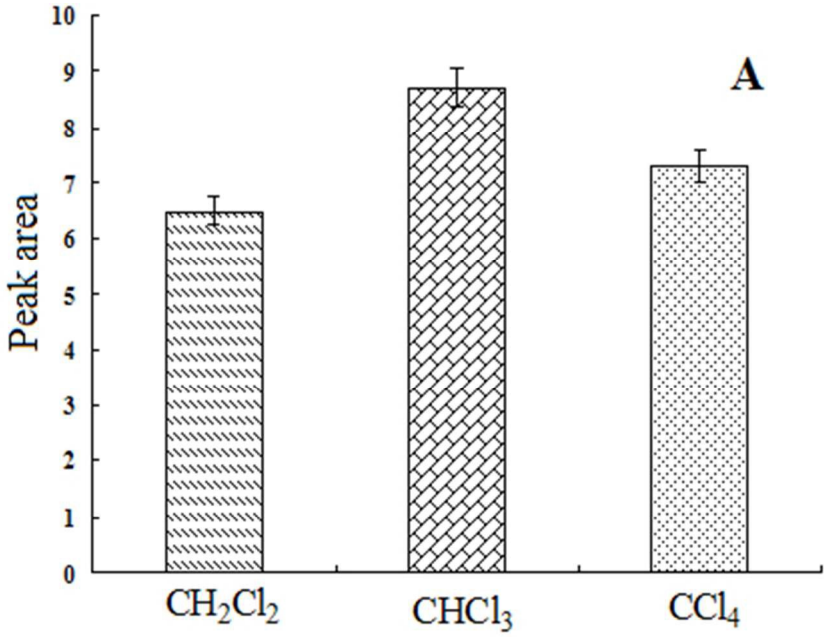

Figure 3A
110x84mm (96 x 96 DPI)

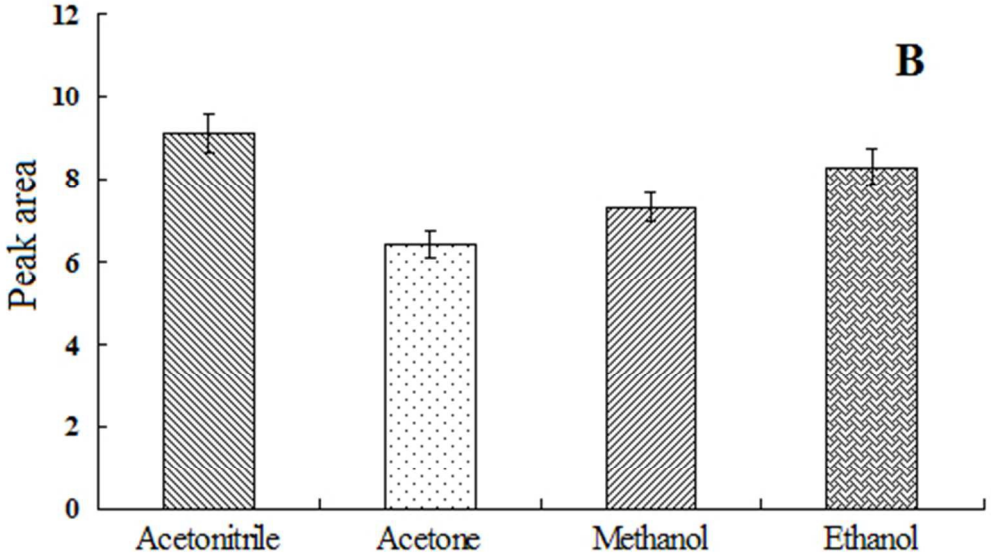

Figure 3B
137x78mm (96 x 96 DPI)

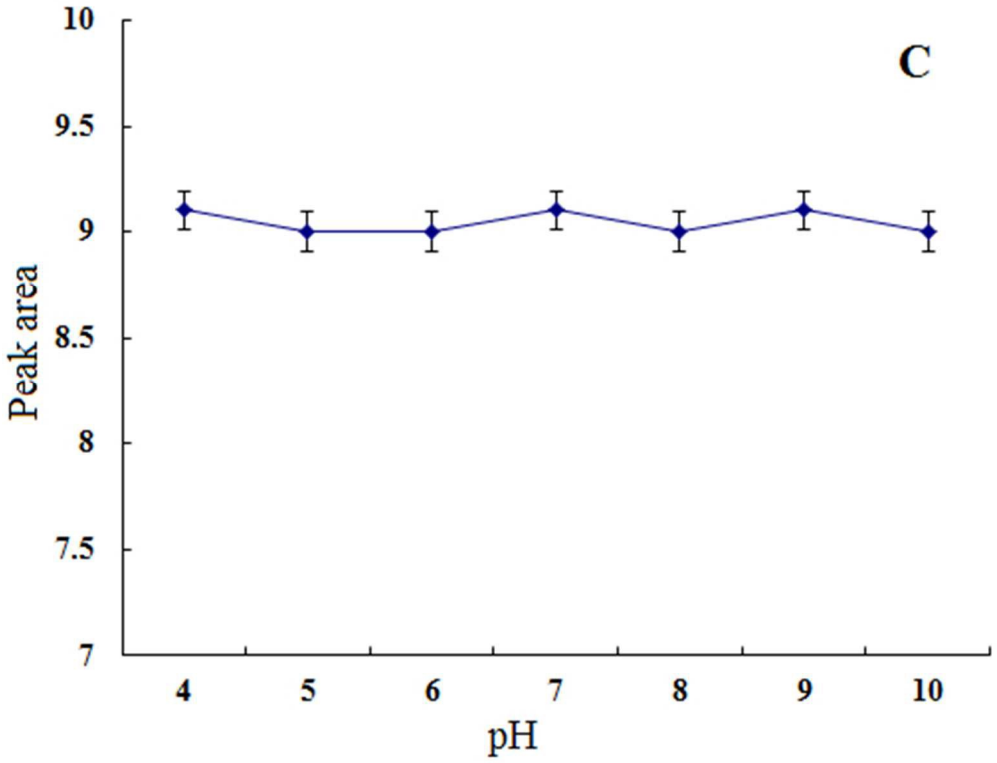

Figure 3C
133x103mm (96 x 96 DPI)

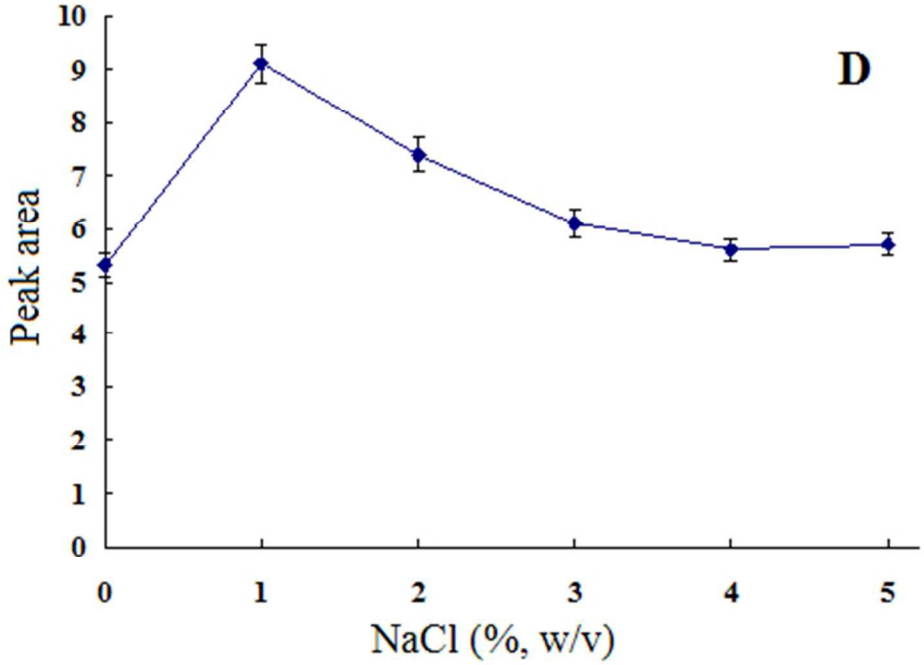

Figure 3D
124x88mm (96 x 96 DPI)

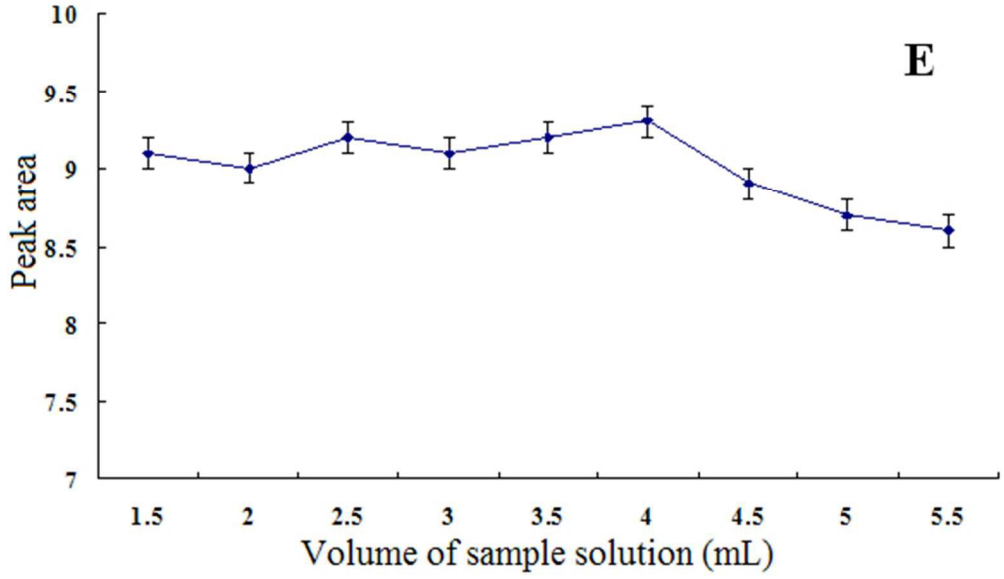

Figure 3E
153x89mm (96 x 96 DPI)

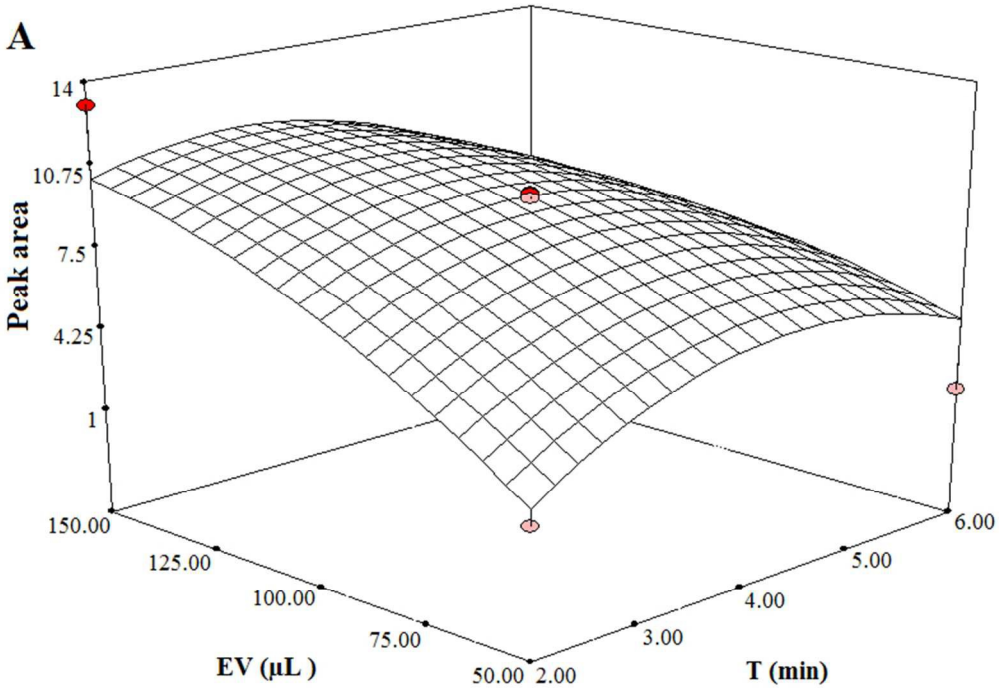

Figure 4A
208x143mm (96 x 96 DPI)

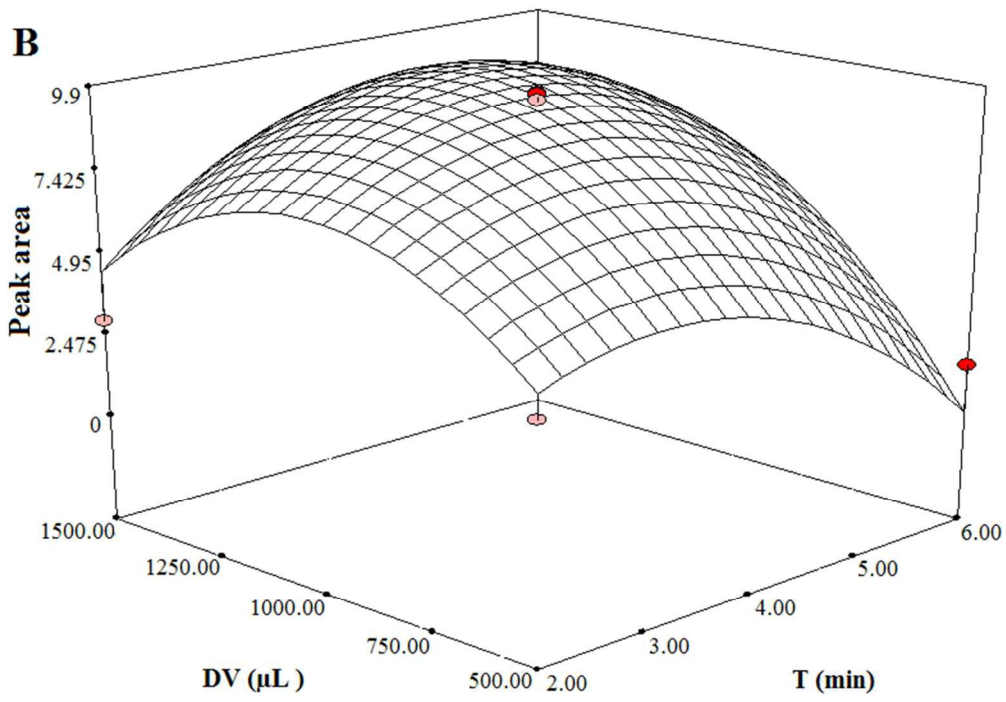

Figure 4B
207x142mm (96 x 96 DPI)

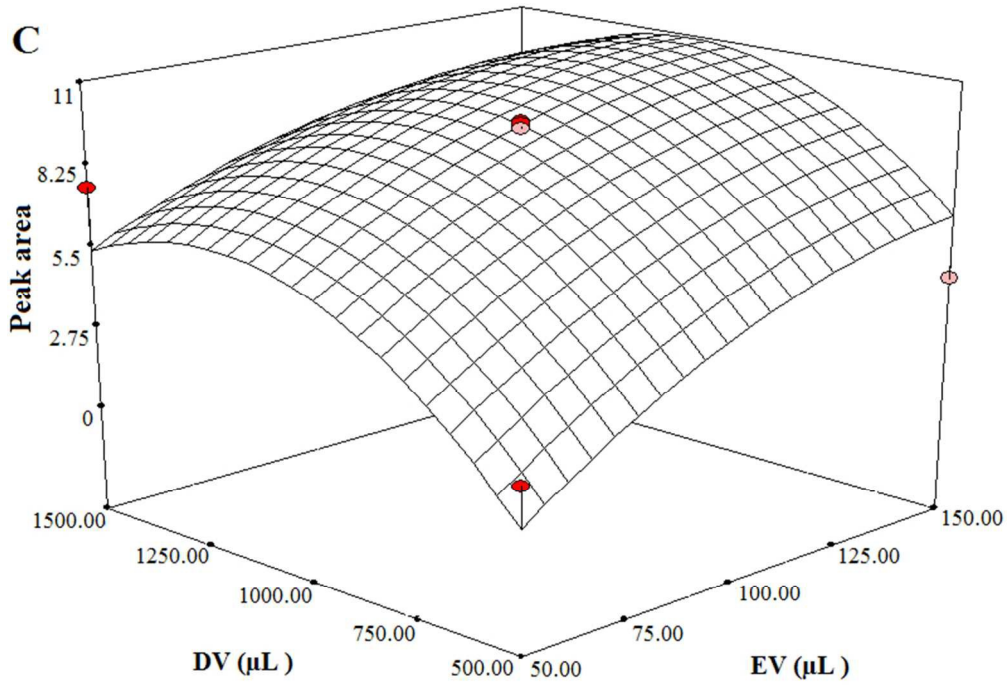

Figure 4C
210x143mm (96 x 96 DPI)