

Field-Amplified Sample Injection and Solid-Phase Extraction for Sensitivity Improvement of Electrophoretic Determination of Indole-3-Acetic Acid and Indole-3-Butyric Acid in Food Samples

Yang Zheng¹ · Xiaofeng Peng¹ · Yiwei Wu¹

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Abstract A sensitive method has been established by using activated carbon as an adsorbent for extraction of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) from bean sprout, tomato, potato, cucumber, and wheat grain prior to field-amplified sample injection-micellar electrokinetic capillary chromatography (FASI-MEKC) detection. Parameters affecting FASI and solid-phase extraction (SPE) were optimized in detail. Under the optimal conditions, the sensitivity of SPE-FASI-MEKC for IAA and IBA was respectively improved by 32.5 and 29.7 times as compared with the direct MEKC. The detection limits were 4.1 for IAA and 5.3 ng mL⁻¹ for IBA with 4.9–5.2% RSD ($n = 9$, $C = 0.03 \mu\text{g mL}^{-1}$). The new method has been successfully applied to detect IAA and IBA in bean sprout, tomato, potato, cucumber, and wheat grain with the recoveries of 81.7–110%.

Keywords Field-amplified sample injection · Indole-3-acetic acid · Indole-3-butyric acid · Micellar electrokinetic capillary chromatography · Solid-phase extraction

Introduction

Plant hormones are a series of trace organic compounds and produced within plant. They are vital links of endogenous

plant development and integrate extracellular signals and play a very important role in regulating and optimizing plant growth and performance at very low levels (Stuepp et al. 2017). With different chemical structures, they serve as different specific and vital functions in plant metabolism, such as senescence, responses to stress, tropism, apical dominance, cell differentiation, cell elongation, and division (Zhang et al. 2017). Hence, a simple and sensitive method for determination of plant hormones in plant samples is significant for understanding of plant growth and physiological function.

At present, methods for detection of plant hormones are high-performance liquid chromatography (HPLC) (Zhang et al. 2017), high-performance liquid chromatography-mass spectrometry (Wang et al. 2017a; Cui et al. 2015; Ding et al. 2013), surface-enhanced raman scattering (Wang et al. 2017b), flow injection analysis fluorimetry (Calatayud et al. 2006), visual colorimetry (Guo et al. 2010), capillary electrophoresis-chemiluminescence (Yin et al. 2010), and micellar electrokinetic capillary chromatography (MEKC) (Sun et al. 2014). Among them, MEKC is an ideal measurement of micro analysis owing to the advantages of speed, efficiency, cheapness, simplicity, high-resolving power, and less sample consumption. However, due to the small size of the capillary sampling and the short UV path, an on-line/off-line preconcentration method has required to improve the sensitive detection of MEKC-UV (Wu et al. 2009). Field-amplified sample injection (FASI), a simple and convenient on-line technology based on the conductivity of sample solutions and electrolyte solutions, has attracted our attentions (Lian et al. 2014). In FASI technology, low conductance sample is required. Therefore, prior to FASI, a sample pretreatment technique like solid-phase extraction is necessary, and adsorbents such as silicon dioxide (Sheikhian and Bina 2016), polymeric molecular materials (Campanella et al. 2016), magnetic nanoparticle (Chang et al. 2017), and multi-walled carbon

✉ Yiwei Wu
chemwuyiwei@163.com

¹ Hubei Collaborative Innovation Center for Rare Metal Chemistry, Hubei Key Laboratory of Pollutant Analysis and Reuse Technology, Institute for Advanced Materials, College of Chemistry and Chemical Engineering, Hubei Normal University, Huangshi 435002, China

nanotube (Wang et al. 2013) have been used to extract plant hormones.

In this paper, activated carbon, a cheap and easy-to-obtain adsorbent, was used to extract indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) in bean sprout, tomato, potato, cucumber, and wheat grain prior to FASI-MEKC analysis. Parameters affecting FASI and solid-phase extraction (SPE) were optimized in detail. A cheap, simple, and sensitive method combined activated carbon SPE with FASI-MEKC has been successfully established to detect IAA and IBA from bean sprout, tomato, potato, cucumber, and wheat grain.

Materials and Methods

Reagents and Solutions

One milligram per milliliter of stock standard solutions of IAA and IBA (structure in Fig. 1) from Guaranteed grade, Aladdin Reagent, Shanghai, China, was individually prepared with methanol (HPLC gradient grade) and stored at 4 °C in the dark. One hundred millimoles per liter of stock standard solutions of sodium dodecyl sulfate (SDS) (Electrophoresis grade, Wuhan Jiangbei chemical reagent factory packaging, Japan) was dissolved in double distilled water. Background electrolyte (BGE) of 25 mmol L⁻¹ borate buffer (pH 9.2) with 12% (v/v) ethanol and 15 mmol L⁻¹ SDS was prepared daily. A series of Britton-Robinson buffer solutions containing H₃PO₄, CH₃COOH, and H₃BO₃ each with concentration at 40 mmol L⁻¹ and double distilled water were used in SPE. All other chemicals, such as HCl, NaOH, trifluoroacetic acid (TFA), and other additives, are of analytical grade. All solutions at a desired concentration were prepared with double distilled water, stored at 4 °C in the refrigerator, filtered through a 0.45- μ m membrane and degassed by ultrasonication for 15 min before use. Five grams of activated carbon (Tianjin Chemical Reagent Co., Ltd.) was immersed in 3.0 mol L⁻¹ HCl and 3.0 mol L⁻¹ NaOH, respectively, washed to neutral with double distilled water, dried in a vacuum, and stored to use.

Instrumentation

A Beckman P/ACE MDQ CE instrument (Fullerton, CA, USA) equipped with an UV detector and uncoated fused-

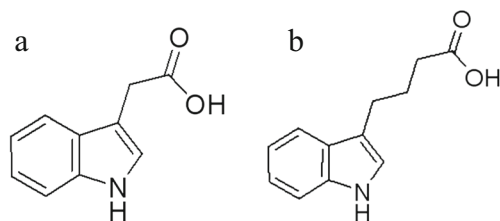


Fig. 1 Chemical structure of **a** IAA and **b** IBA

silica capillary tubing of 51 cm (43 cm to the detector, 75 μ m id \times 375 μ m od) with a polyimide outer coating (Yongnian Optical Fiber, Hebei, China) was used to FASI-MEKC experiments. In optimization of SPE conditions, determination of IAA and IBA was conducted on an U-3010 spectrophotometer (Hitachi, Japan). The pH measurements were performed by a PB-10 exact digital pH meter (Sartorius, Germany). A SK2200H type ultrasonic cleaning instrument (Shanghai Secco Ultrasonic Instrumental, Shanghai, China) and an 80-1 type centrifuge (Changzhou Guohua, Jiangsu, China) were used to pretreat the real sample.

Experimental

Procedure for MEKC

MEKC conditions were based on literature (Chen et al. 2011) with a slight modification. All new capillaries were rinsed sequentially with ethanol, 1.0 mol L⁻¹ HCl, 1.0 mol L⁻¹ NaOH, and double distilled water for 10 min and then equilibrated with BGE for 10 min. Before runs, the capillary was swilled out with ethanol, double distilled water, and BGE for 1, 2, and 4 min in sequence. Hydrodynamic injection was carried out at 0.5 psi for 15 s. All MEKC runs were implemented at 25 kV and 214 nm. The procedure was controlled by a Beckman 32 Karat 7.0 Software System.

Procedure for FASI-MEKC

A water plug was introduced into the capillary with hydrodynamic injection for 15 s at 0.5 psi. A high voltage (10 kV) was then applied to electrokinetically introduce the sample into the capillary for 70 s. When both electrode reservoirs were filled with BGE, the separation of IAA and IBA was performed by supplying 25 kV across the capillary.

Procedure for Activated Carbon SPE

Fifteen milligrams of activated carbon was added to 10 mL sample solution at pH 3.5, mixed, ultrasonicated for 15 min, and centrifuged at 3500 rpm for 10 min. Plant hormones retained on activated carbon were eluted with 1.0 mL methanol-acetonitrile mixture (v/v 10:1). Plant hormones in eluent were collected and dried in the vacuum for 5 h at 30 °C, then dissolved in 10 μ L methanol, and finally diluted to 1.0 mL with 0.1% (v/v) TFA prior to FASI-MEKC analysis.

Real Sample Preparation

Three grams of bean sprout, tomato, potato, cucumber, and wheat grain was accurately weighed, cut into pieces, ground, respectively, dissolved in 10 mL of 80% methanol,

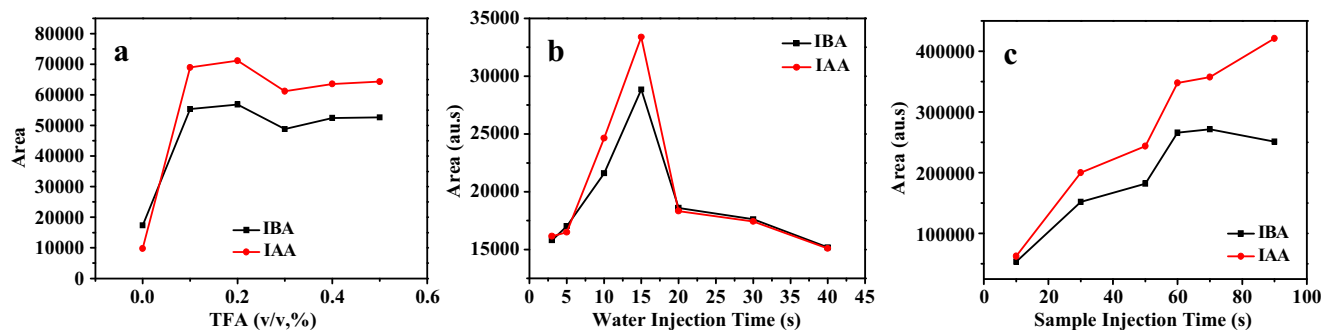


Fig. 2 Effects of TFA content in sample matrix (a), water plug length (b), and sampling injection time (c) on detection sensitivity. Sample conditions: $20.0 \mu\text{g mL}^{-1}$ IAA and IBA in 0.1% (v/v) TFA (for b and c). MEKC conditions are listed in Table 1. FASI conditions: pressure

injection of water for 10 s for a and 15 s for c at 0.5 psi followed by electrokinetic injection of sample for 40 s for a and 20 s for b both at 10 kV

ultrasonicated for 15 min, and placed overnight in a refrigerator at 4°C . Then, the mixture was filtrated through a $0.45\text{-}\mu\text{m}$ membrane filter. The filtrate was collected, dried in a vacuum overnight at 30°C and dissolved in 1.0 mL methanol, and stored for use

Results and Discussion

Optimization of FASI Conditions

To improve the detection sensitivity of MEKC, an on-line preconcentration method, FASI, was tried. Since analytes, which can be separated successfully by MEKC, are not always suitable for FASI-MEKC, we should, in a univariate way, study that IAA and IBA can both be detected by FASI-MEKC. According to the principles of FASI, parameters affecting FASI, such as sample matrix, concentration of NaCl, water plug length, sample injection time, and voltage, were researched one by one to get the best discrepancy of electrical resistivity between background electrolyte and sample solution.

Effect of TFA

Presence of acid in sample matrix can promote protonation of IAA and IBA and hence improve the stacking efficiency of

FASI. Although TFA, formic acid, phosphoric acid, and acetic acid were all used for FASI (He et al. 2013; Xu et al. 2006; Bernad et al. 2011), TFA is the widest one due to the highest proton-donating capability (TFA > phosphoric acid > formic acid > acetic acid) (Sun and Wu 2013). Hence, TFA was chosen and investigated at a range of 0.1–0.5% (v/v). The results in Fig. 2a show that the stacking efficiency increases with TFA, but when its content is over 0.2% (v/v), the peak shape became broader. It is an attribute to a higher TFA content causing a lower pH and a bigger conductivity of the sample solution, resulting in an insufficient stacking. Thus, 0.1% (v/v) TFA was selected.

Effect of Organic Solvent

To reduce the conductivity and enhance the stacking efficiency, organic additives were usually added into the matrix (Yang et al. 2006). Effects of common organic additives, such as methanol, acetonitrile, cyclodextrin, 2-(2-[4-(1,1,3,3-tetramethylbutyl) phenoxy]ethoxy)ethanol, and alkylphenol ethoxylates, were investigated, respectively. The results showed that both methanol and acetonitrile could not improve the enrichment efficiency; however, cyclodextrin, 2-(2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy)ethanol and alkylphenol ethoxylates decreased the separation efficiency of IAA and IBA. Therefore, no organic additive is adopted.

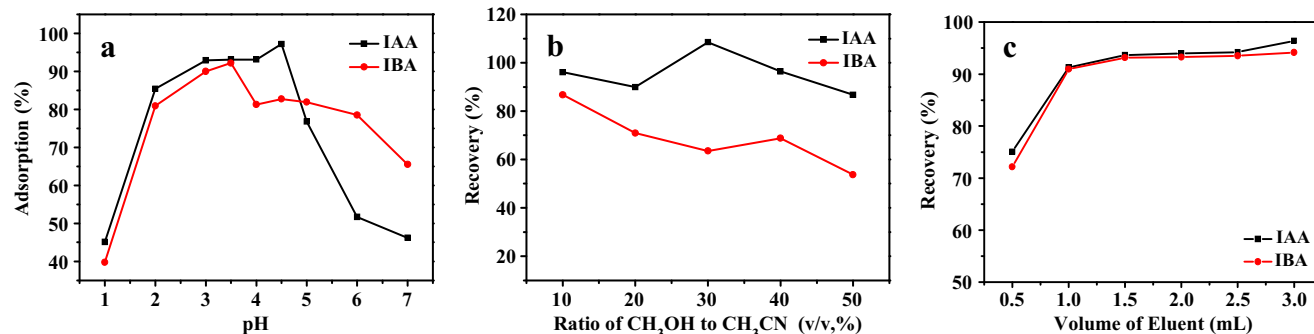


Fig. 3 Effect of pH on adsorption (a), effect of CH_3OH -to- CH_3CN ratio on desorption (b), and effect of eluent volume on recovery (c). Twenty micrograms per milliliter of IAA and IBA for a, b, and c

Table 1 Experimental optimized parameters of SPE-FASI-MEKC

| | |
|-----------------------------------|--|
| MEKC conditions | |
| Capillary characteristics | 51 cm total length with effective length 43 cm (75 μm id \times 375 μm od) |
| Running buffer | 25 mmol L ⁻¹ borate buffer containing 15 mmol L ⁻¹ SDS and 12% ethanol (v/v) at pH 9.2 |
| Voltage | + 25 kV |
| Injection | Pressure injection for 15 s at 0.5 psi |
| Detection wavelength | 214 nm |
| FASI conditions | |
| Sample matrix | 0.1% (v/v) TFA |
| Water plug length | Pressure injection for 15 s at 0.5 psi |
| Sample injection time and voltage | Electrokinetic injection for 70 s at + 10 kV |
| SPE conditions | |
| Adsorption conditions | 0.04 mol L ⁻¹ Britton-Robinson buffer at pH 3.5 for 15 min |
| Elution conditions | 1.0 mL of 10:1 ratio of methanol to acetonitrile for 15 min |
| Sample volume | 30 mL |
| Adsorption capacity | 43.6 mg g ⁻¹ for IAA, 39.3 mg g ⁻¹ for IBA |

Effect of NaCl

Difference conductivity between the running buffer and sample solution is a key factor for FASI. The lower conductivity the sample solution has, the higher sensitivity FASI-MEKC will (Yan et al. 2009). Therefore, influence of NaCl (0–1.0% (m/v)) on stacking efficiency was investigated. The results revealed that the sensitivity decreased when the salt concentration was greater than 0.8%. In this work, the salt level in bean sprout and tomato after through sample pretreatment and activated carbon SPE is lower than 0.8% (m/v).

Optimization of Water Plug Length

In FASI, it has been proved that preinjection of a short water plug before sampling can improve the stacking efficiency (Yang et al. 2006; Quirino and Terabe 2000). However, the total amount of IAA and IBA introduced into the capillary by FASI will become less with increasing length of water plug.

Hence, to achieve the best stacking efficiency, the length of the water plug should be kept as short as possible. As illustrated in Fig. 2b, an optimal water plug length is 15 s (hydrodynamic injection of water at 0.5 psi).

Effect of Sampling Injection Voltage and Time

Influences of sampling injection voltage (2–11 kV) and time (10, 30, 50, 60, 70, 90 s) on the stacking efficiency were investigated, respectively. When the sampling voltage increased from + 2 to + 10 kV, the migration time became short, the peak area increased; however, when the voltage continued to increase, the Joule heat and background noise also increased. As a result, an injection voltage of + 10 kV, which gave rise to an efficient sample stacking, was selected. Figure 2c shows the effect of injection time. As seen, the peak areas increase with the injection time from 10 to 70 s, but over 70 s, the overloaded samples lead to decrease of separation efficiency. Therefore, 70 s was selected.

Fig. 4 Electropherograms of 10.0 $\mu\text{g mL}^{-1}$ standard IAA and IBA obtained by (a) direct MEKC, (b) FASI-MEKC, and (c) SPE-FASI-MEKC. The conditions are as in Table 1

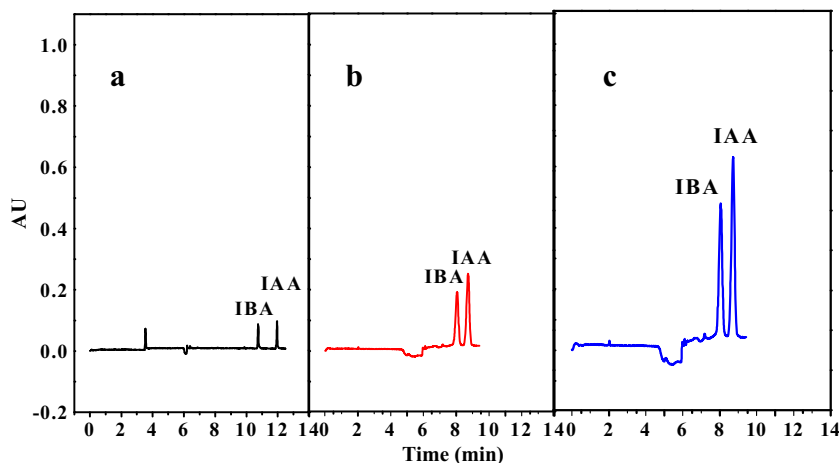


Table 2 Figures of merit of SPE-FASI-MEKC

| Analytes | Regression equation | Correlation coefficient | Linear range ($\mu\text{g mL}^{-1}$) | RSD (%) ^a | EF ^b | Detection limits ($\mu\text{g mL}^{-1}$) |
|----------|---------------------------------|-------------------------|--|----------------------|-----------------|--|
| IAA | $y = 1.49 \times 10^4 x + 15.3$ | 0.9952 | 0.03–40 | 5.2 | 32.5 | 0.0041 |
| IBA | $y = 1.21 \times 10^4 x + 29.1$ | 0.9948 | 0.03–40 | 4.9 | 29.7 | 0.0053 |

^a $C = 0.03 \mu\text{g mL}^{-1}$, $n = 9$

^b Enhancement factor

Optimization of SPE Conditions

Effect of pH

To characterize the effect of pH on activated carbon retaining IAA and IBA, pH 1.0–7.0 controlled with Britton-Robinson buffer was tested and the experimental results are shown in Fig. 3a. As seen, IAA can be quantitatively adsorbed at pH 3.0–4.5, and both IAA and IBA can be quantitatively extracted at pH 3.5.

Effect of Adsorption Time

Adsorptive time is a factor affecting analytical speed. Thus, adsorptive time of IAA and IBA at 5, 10, 15, 20, and 25 min was investigated, respectively. The results showed that quantitative adsorption rates of IAA and IBA were obtained when the adsorption time was more than 15 min. Therefore, an optimum adsorption time of 15 min was selected.

Effect of Elution Conditions

With respect to stripping of IAA and IBA from activated carbon, effects of various eluents including methanol, acetonitrile, acetone, ethyl acetate, and mixture of methanol and

acetonitrile (*v/v*) were studied, respectively. The results showed that IAA and IBA could be desorbed quantitatively only by the mixture of methanol and acetonitrile (*v/v*). Thus, the volume ratio of methanol to acetonitrile was further tested and the results are displayed in Fig. 3b. As seen, quantitative recoveries for IAA and IBA are reached at 10:1 ratio of methanol to acetonitrile. Therefore, a 10:1 ratio of methanol to acetonitrile was adopted.

Influence of elution time (10–30 min) on recoveries of IAA and IBA was investigated; the results showed that the recoveries of IAA and IBA could reach 90% at 15 min. Meanwhile, the effect of elution volume (0.5–3.0 mL) was also optimized and the experimental results are shown in Fig. 3c. It is clear that both IAA and IBA can be recovered quantitatively when the volume is over 1.0 mL. Therefore, 15 min and 1.0 mL were chosen as the elution time and volume.

Effect of Sample Volume

In order to assess the possibility of enriching trace IAA and IBA from a large volume, the effect of sample volume on IAA and IBA recovery was studied. Hence, 10–35 mL of sample solutions containing 0.1 mg IAA and IBA were respectively carried out according to the recommended procedure. Up to a sample volume of 30 mL, quantitative recovery (> 90%) was

Table 3 Comparison of the proposed method with reported methods

| Sample | Analytes | Methods | LODs ($\mu\text{g mL}^{-1}$) | EF | Ref. |
|--|----------|----------------|--------------------------------|--------------|-------------------------|
| Arabidopsis, tomato, pear, cucumber, and ginger | IAA | dSPE-HPLC-UV | 0.05 | – | Zhang et al. 2017 |
| | IBA | | 0.03 | | |
| Pea plants | IAA | SPE-HPLC-UV | 0.16 | 100 4000 | Sheikhian and Bina 2016 |
| | IBA | | 0.091 | | |
| Grapes | IAA | SPE-CE-UV | 80.583 89.426 | – | Li et al. 2012 |
| Wheat | IAA | SPE-FASS-CE-UV | 0.008 | – | Yang et al. 2016 |
| | IBA | | 0.025 | | |
| Bean sprout | IAA | dCPE-CE-ECL | 0.438 | 40.5 43.4 | Yin et al. 2010 |
| | IBA | | 0.569 | | |
| Banana | IAA | ASEI-CE-UV | 0.00067 | 36.2 | Huang et al. 2014 |
| Bean sprout, tomato, potato, cucumber, and wheat grain | IAA | SPE-FASI-CE-UV | 0.0041 | 32.5 29.7 | This work |
| | IBA | | 0.0053 | | |

FASS field-amplified sample stacking, CPE cloud point extraction, ECL electrochemiluminescence, ASEI anion-selective exhaustive injection

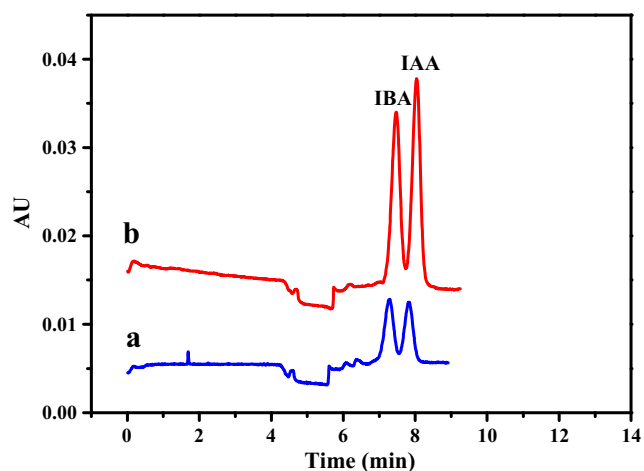


Fig. 5 SPE-FASI-MEKC analysis of IAA and IBA in mung bean sprout. (a) Unspiked solution and (b) spiked solution. Conditions are as in Table 1

obtained, indicating activated carbon SPE has a good enrichment ability.

Static Adsorptive Capacity

Using a pH 3.5 and a 15-min stirring time as the adsorptive condition, a 15-mg portion of activated carbon was shaken with 30.0 mL aqueous solution containing 0.01–0.025 mg mL⁻¹ of IAA and IBA. After the distribution equilibrium was reached, the concentration of IAA and IBA in

solution was determined by UV-vis absorption spectra at 214 nm. The results showed that the static adsorptive capacities of activated carbon for IAA and IBA were 43.6 and 39.3 mg g⁻¹, respectively.

Analytical Performance

Under the optimal conditions (Table 1), Fig. 4 shows the electropherograms of IAA and IBA by direct MEKC, FASI-MEKC, and SPE-FASI-MEKC. By comparing the peak intensity of the three electropherograms, obvious enhancements in sensitivity are observed, indicating SPE and FASI have remarkable preconcentration ability. The enhancement factors for IAA and IBA are 32.5 and 29.7 times, respectively, (enhancement factor $(A/C)/(A_0/C_0)$ where A and A_0 are the peak areas of the analyte under SPE-FASI and normal conditions, respectively; C and C_0 are analyte concentrations under SPE-FASI and normal conditions, respectively).

Analytical characteristic data of the developed SPE-FASI-MEKC method are listed in Table 2. The calibration curves are established using peak area versus concentration. The linear ranges of IAA and IBA are 0.03–40 µg mL⁻¹. LODs, calculated as the concentration of analytes that give rise to peak areas with three times of signal-to-noise ratio (S/N), are 4.1 and 5.3 ng mL⁻¹ for IAA and IBA, and RSD are 4.9 to 5.2% ($n = 9$, $C = 0.03$ µg mL⁻¹), respectively.

Table 3 compares the analytical characteristics of this method with the others. It is obvious that LODs of IAA and IBA in

Table 4 Analytical results of IAA and IBA in food samples

| Sample | Added (µg mL ⁻¹) | IAA | | IBA | |
|------------------|------------------------------|--|--------------|--|--------------|
| | | Measured ^a (µg mL ⁻¹) | Recovery (%) | Measured ^a (µg mL ⁻¹) | Recovery (%) |
| Mung bean sprout | 0 | 0.45 | – | 0.38 | – |
| | 0.50 | 0.89 ± 0.06 | 88.2 | 0.80 ± 0.14 | 84.3 |
| | 1.0 | 0.97 ± 0.13 | 104.3 | 1.35 ± 0.07 | 98.6 |
| Tomato | 0 | 0.37 | – | 0.29 | – |
| | 0.50 | 0.78 ± 0.05 | 81.7 | 0.76 ± 0.11 | 93.5 |
| | 1.0 | 1.29 ± 0.07 | 91.8 | 1.39 ± 0.08 | 110.3 |
| Potato | 0 | ND | – | ND | – |
| | 0.50 | 0.47 ± 0.02 | 94.0 | 0.48 ± 0.03 | 96 |
| | 1.0 | 0.96 ± 0.11 | 95.8 | 0.98 ± 0.13 | 97.5 |
| Cucumber | 0 | 0.13 | – | 0.21 | – |
| | 0.50 | 0.66 ± 0.05 | 106 | 0.71 ± 0.1 | 100 |
| | 1.0 | 1.15 ± 0.09 | 102 | 1.12 ± 0.06 | 91.0 |
| Wheat grain | 0 | ND | – | 1.27 | – |
| | 0.50 | 0.45 ± 0.03 | 90.0 | 1.76 ± 0.12 | 98.0 |
| | 1.0 | 0.92 ± 0.06 | 91.5 | 2.31 ± 0.09 | 104 |

ND not detected

^a For three determinations

this method are lower than those in studies by Zhang et al. (2017), Sheikhan and Bina (2016), Li et al. (2012), Yang et al. (2016), and Yin et al. (2010) and higher than those by Huang et al. (2014). Meanwhile, FASI, just based on the discrepancy of electrical resistivity between the sample solution and background electrolyte, is more convenient and simpler. As for HPLC (Zhang et al. 2017; Sheikhan and Bina 2016), the operation costs are much more expensive than FASI-MEKC-UV. All of these indicate this work is a sensitive one besides simplicity and low cost.

Analytical Application

The proposed method was applied to determination of IAA and IBA in bean sprout, tomato, potato, cucumber, and wheat grain under the optimum conditions. Real samples and their spiked solutions were treated as in the “**Real Sample Preparation**” section. Figure 5 shows the electropherogram of IAA and IBA in bean sprout by SPE-FASI-MEKC. In Fig. 5, (a) is an unspiked sample solution; (b) is a spiked sample solution. The analytical results listed in Table 4 show that the recoveries of IAA and IBA are 81.7–110%, indicating the proposed method is suitable and acceptable.

Conclusion

A simple, sensitive, and low-cost method for determination of IAA and IBA in bean sprout, tomato, potato, cucumber, and wheat grain has been successfully established based on SPE and FASI-MEKC. The strategy of coupling SPE with FASI results in improving the sensitivity of MEKC by 32.5 and 29.7 times in determination of IAA and IBA, respectively. Under the optimum conditions, LODs of IAA and IBA are as low as 4.1 and 5.3 ng mL⁻¹, respectively, which are comparable to or better than those of methods (Table 3).

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Compliance with Ethical Standards

Conflict of Interest Yang Zheng declares that he has no conflict of interest. Xiaofeng Peng declares that he has no conflict of interest. Yiwei 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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