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Simultaneous determination of phytohormones containing carboxyl in crude extracts of fruit samples based on chemical derivatization by capillary electrophoresis with laser-induced fluorescence detection

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ABSTRACT

An efficient and sensitive capillary electrophoresis with laser-induced fluorescence detection (CE–LIF) method has been developed for the simultaneous determination of phytohormones containing carboxyl group, including gibberellic acid, indole-3-acetic acid, abscisic acid, jasmonic acid, indole butyric acid, 1-naphthalene acetic acid and 2,4-dichloro-phenoxy acetic acid, based on the chemical derivatization with 6-oxy-(acetypiperazine) fluorescein. Using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide as the condensing reagent, the derivatization reaction completed at 60 °C in 60 min and the derivatization limits could reach 20 nmol L^{-1}. The formed derivatives of seven phytohormones have been separated and quantified within 20 min. The linearity was found in the range of 0.01–1 μmol L^{-1} and the limits of detection were 1.6–6.7 nmol L^{-1} (S/N = 3). The proposed method has been applied to analyze the crude extract of 0.5 g banana samples directly without further purification and the recoveries varying from 90.7 to 106.1%.

1. Introduction

Phytohormones play key roles in various physiological processes of plants, such as cell division, cell differentiation, apical dominance, shoot development, induction of photosynthesis gene expression, and retarding leaf senescence [1–5]. For examples, gibberellic acids (GA_3) are a class of phytohormones that have profound and diverse effects on plant growth and development [2,3]. As a couple of phytohormones with opposite biological functions, indole-3-acetic acid (IAA) stimulates growing processes and abscisic acid (ABA) controls plant senescence [2]. Jasmonic acid (JA) acts as a signal molecule in plant defense systems responding to various biotic and abiotic stresses involving mechanical wounding as well as herbivore, bacterial and fungal pathogen attacks [4–6]. Besides these endogenous phytohormones, some low-cost exogenous phytohormones including indole butyric acid (IBA), 1-naphthalene acetic acid (NAA) and 2,4-dichloro-phenoxy acetic acid (2,4-D), have been applied widely as plant growth regulators to get high yield in agriculture [7,8].

Phytohormones exert their functions additively, synergistically, or antagonistically and their regulation is dependent greatly on their levels in plant tissues [9,10]. Therefore, in order to understand a variety of physiological processes in plants and cross-talk among phytohormones, the development of sensitive and simultaneous determination method for multiple classes of phytohormones is of significant biological importance.

For this purpose, gas chromatography (GC), liquid chromatography (LC) and capillary electrophoresis (CE) coupled to mass spectrometry, UV detection or fluorescence detection have been proposed and demonstrated as powerful tools for the analysis of multi-phytohormones [11–16]. Mass spectrometry-based methods are the most important and effective method in analysis of phytohormones. By coupling with SPME as a standard step in complex matrix sample preparation, both qualitative and quantitative analysis of various phytohormones can be achieved sensitively [17]. But while LC–MS adopt in routine use for quantitative analysis, the expensive isotope internal standard is necessary [17]. As mature and reliable coupled detection techniques in routine use, ultraviolet or fluorescence detection is relatively cheap and convenient. In all these methods, capillary electrophoresis with laser-induced fluorescence (CE–LIF) detection is most sensitive, which is much preferable to the analysis of trace phytohormones simultaneously in small sample volume.

Commonly, chemical derivatization with a proper derivatizing reagent for phytohormones is always indispensable in these cases since most of phytohormones do not have high chro-

Abbreviations: APF, 6-oxy-(acetypiperazine) fluorescein; GA_3, gibberellic acid; IAA, indole-3-acetic acid; ABA, abscisic acid; JA, jasmonic acid; IBA, indole butyric acid; NAA, 1-naphthalene acetic acid; 2,4-D, 2,4-dichloro-phenoxy acetic acid; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; SDS, sodium dodecyl sulphate; SPE, solid phase extraction.

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mophore or fluorophore to be detected [19,20]. Further more, besides a highly fluorescent fluorophore and a selectively reactive moiety, a good derivatizing reagent used in CE–LIF should also have an excitation wavelength which matches the wavelength of commercial LIF detection system. Therefore, up till now, only a few derivatizing reagents have been applied in CE–LIF detection for phytohormones, such as 3-(2-furoyl)quinoline-2-carboxaldehyde (FQ) [21], 8-aminopyrene-1,3,6-trisulfonate [22], 9-anthryldiazomethane and 5-bromomethyl fluorescein [23,24]. Moreover, these works focused on the determination of single phytohormone, which did not represent entirely the advantages of CE separation.

6-Oxy-(acetyl piperazine) fluorescein (APF) is a new derivatizing reagent for carboxyl compounds synthesized in our group [25]. As a fluorescein-based label, it has high fluorescence quantum yield (0.41 in pH9.0 Na_2HPO_4–H_3Cit_3 buffer) and suitable wavelength ($\lambda ex/\lambda em = 467/512$ nm) compatible with the argon ion LIF detector. In this paper, a CE–LIF method to simultaneously quantify multiple classes of phytohormones in crude extract of banana samples has been developed based on APF derivatization. The limits of detection could reach 1.6–6.7 nmol L^{-1}. The potential of the proposed method has been well confirmed by the evaluation of analytical performance and the analysis of practical samples.

2. Experimental

2.1. Chemicals

Unless otherwise specified, all reagents used were of analytical grade. GA_3, IAA, ABA, JA, IBA, NAA, 2,4-D, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and sodium dodecyl sulphate (SDS) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Boric acid and sodium borate were purchased from Shanghai Chemicals Company (Shanghai, China). APF was synthesized in our laboratory.

1.0×10^{-3} mol L^{-1} of APF, 1.0×10^{-3} mol L^{-1} of each phytohormone standard solution and 1.0×10^{-2} mol L^{-1} of EDC were prepared with anhydrous acetonitrile. All aqueous solutions were prepared with deionized water purified by a Millipore-Q system (Millipore, Bedford, MA, USA). Borate buffer solutions were obtained by mixing boric acid solution and sodium borate solution to the required pH value. The running buffer was prepared with borate buffer, 0.1 mol L^{-1} SDS, ethanol and water.

2.2. Apparatus

CE experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis system (Beckman, Fullerton, CA, USA) with an LIF detector. An argon ion laser (3 mW) was used as excitation source (488 nm) and the electropherograms were recorded at 520 nm emission wavelength. All separations were performed in an uncoated fused-silica capillary (Yongnian, Optic Fiber, Hebei, China) 60.2 cm in length (effective length of 50 cm) and 75 μm i.d. The pH value of solution was measured using a Delta 320 pH meter (Mettler-Toledo, Shanghai, China).

2.3. Derivatization procedure

An appropriate volume of the mixed phytohormone standard solution, 90 μL of 1×10^{-2} mol L^{-1} EDC and 18 μL of 1×10^{-3} mol L^{-1} APF were transferred into a 1.5 mL vial. The whole solution was diluted to 200 μL with acetonitrile and then the well-mixed solution was kept at 60 °C for 60 min in dark. Before analysis, 40 μL of the resulting mixture was diluted to 200 μL with the electrophoresis buffer.

2.4. Capillary electrophoresis procedures

Prior to use, each new capillary was conditioned by rinsing with methanol, H_2O, 1 mol L^{-1} HCl, H_2O, 1 mol L^{-1} NaOH, and H_2O for 30 min in order. The instrument was programmed to rinse the capillary with H_2O, 0.1 mol L^{-1} NaOH, H_2O, 0.1 mol L^{-1} HCl, and H_2O for 5 min in succession daily. Before runs, the capillary was swilled out with 0.1 mol L^{-1} HCl followed H_2O and running buffer for 3 min each. And all the rinse programs above were performed at 25 °C. The running buffer consisted of 25 mmol L^{-1} pH 9.20 borate buffer, 15 mmol L^{-1} SDS and 12% ethanol (v/v). Before use, the running buffer was filtered using a 0.22 μm nylon membrane filter and degassed in an ultrasonic for 5 min. Sample introduction was performed by pressure of 0.5 psi for 5 s from the anodic side. Analyses were performed at 25 °C using a liquid coolant in a sealed cartridge with a running voltage of 22.5 kV.

2.5. Sample preparation

Sample preparation was according to the reference [21] with slight changes. Banana samples were purchased from local market. About 0.5 g of banana was ground into fine powder in the presence of liquid nitrogen and transferred into a vial filled with 10 mL of cold acetonitrile. After high-speed blender, the mixture was maintained overnight at 4 °C, and then centrifuged at 4 °C (10,000 × *g* for 5 min). 200 μL of the supernatant was derivatized with APF as the above derivatization procedure.

The recoveries of phytohormone analysis were obtained by adding 2.5×10^{-7} mol L^{-1} GA_3, and 1.5×10^{-7} mol L^{-1} of other standard phytohormones with standard solution in the crude extract.

3. Results and discussion

GA_3, IAA, ABA and JA are the chief representatives of gibberellins, auxines and inhibitors, respectively. IBA, NAA and 2,4-D are the most widely used auxines as plant growth regulators in agriculture. Meanwhile, there is a carboxyl in their structure, which provides a suitable reactive group for APF labeling. Therefore, the feasibility of APF in the determination of GA_3, IAA, ABA, JA, IBA, NAA and 2,4-D with CE–LIF has been assessed by the optimization of separation and derivatization conditions, and analysis of biological samples.

3.1. Separation of APF derivatives

Since the derivatives of plant hormones with APF are charged, capillary zone electrophoresis (CZE) was initially used for the separation. However, nearly all the fluorescent substances were flushed together when pH value of the electrolyte was above 9.00. The peaks trended to be separated with the decrease of pH. Since the lower pH value brought about the lower fluorescence of derivatives due to the presence of fluorescein fluorophore, it was not desirable to reduce the electrolyte pH further for the separation of the plant hormone derivatives in CZE mode. Therefore, micellar electrokinetic capillary chromatography (MECC) was introduced for the separation.

For MECC mode, SDS, the most popular anionic surfactant, was used. The concentration of SDS is a critical parameter in MECC separation. Since the critical micelle concentration of SDS is about 8 mmol L^{-1}, the effect of SDS concentration on the separation was studied from 10 to 20 mmol L^{-1}, using 25 mmol L^{-1}, pH 9.0 borate buffer as the background electrolyte. Although the resolution of the analytes was improved, GA_3–APF derivatives and the reagent, as well as IBA and NAA derivatives, co-migrated. The addition of 12.5 mmol L^{-1} of SDS could obtain relatively good resolution and

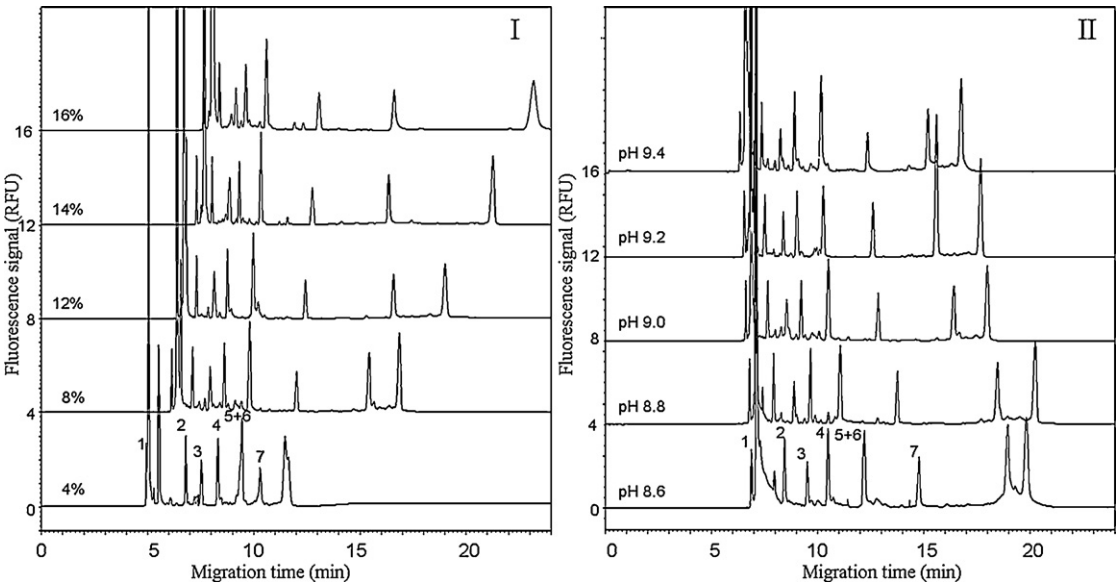

Fig. 1. Electropherogram obtained from MECC mode (I) in different concentration of ethanol and (II) in different pH. Running buffer: 25 mmol L^{-1} borate buffer, 12.5 mmol L^{-1} SDS; capillary: 65 cm (50 cm effective length) × 75 μm i.d.; injection: 5 s at 0.5 psi; separation voltage: 22.5 kV; temperature: 25 °C. Peaks: (1) GA_3; (2) IAA; (3) ABA; (4) JA: (5) IBA; (6) NAA; (7) 2, 4-D.

sharp peaks of the analytes, which was chosen as a temporarily condition for further optimization.

It has been reported that the use of an organic modifier to the CE running buffer is an important parameter to improve the resolution because it increases the hydrophobicity of the mobile phase and thus change the distribution of analytes between micellar pseudophase and mobile phase [26]. Since acetonitrile is a protophobic dipolar aprotic solvent and has significantly different physico-chemical properties from water and methanol [27], acetonitrile was adopted as the organic modifier firstly. It was found that the peaks became broader and the separation became worse with the increase of acetonitrile from 0 to 20% (v/v) in the electrolyte containing 25 mmol L^{-1} pH 9.2 boric acid/sodium borate buffer and 12.5 mmol L^{-1} SDS. Accordingly, ethanol was investigated at 4–16% (v/v) as an alterative for it may causes changes in viscosity and dielectric constant and, consequently, in the zeta potential. As shown in Fig. 1-I, the addition of ethanol in the running buffer made the resolution higher obviously. The peaks of GA_3–APF derivatives and the reagent were separated, while the peaks of IAA–APF derivatives and the reagent overlapped when ethanol content was more than 14%. In the test range, IBA and NAA derivatives still co-migrated throughout. As a result, 12% (v/v) ethanol was selected as the optimum organic modifier.

The pH value of running buffer can adjust the velocity of EOF and the acidic/alkaline equilibrium of analytes, and influence the intrinsic mobility, water-solubility of the analytes and the partition coefficients into the micellar phase in MECC separation mode. Considering together with the pH-dependent fluorescence of fluorescein fluorophore, the effect of pH value of running buffer on the separation was examined in the range of 8.6–9.4, which is shown in Fig. 1-II. In this pH range, the phytohormone–APF derivatives were partially negatively charged due to the presence of the carboxylic group in APF molecule. It was observed that the migration times for most analytes decreased slightly with the increasing pH. However, the peaks of IBA and NAA derivatives still overlapped. When the pH value was lower than 9.2, the tailing of the reagent peaks was obvious. Thus, pH 9.2 was used.

The effect of the buffer concentration in the range 15–25 mmol L^{-1} on the separation has also been investigated. With the increase of buffer concentration, the tailing of the peaks was prevented effectively as the higher ionic strength of the buffer attenuated the double layer of the inner capillary wall and accordingly suppressed EOF. At the same time, longer migration time and peak expansion became other problems. Therefore, 25 mmol L^{-1} was used as the optimized buffer concentration for it provided a suitable compromise.

Based on the electrolyte containing 25 mmol L^{-1} pH 9.2 boric acid/sodium borate buffer and 12% (v/v) ethanol, the effect of SDS concentration around 12.5 mmol L^{-1} from 10 to 17.5 mmol L^{-1} on the separation has been investigated in detail. It can be seen from Fig. 2-I that the complete resolution of IBA and NAA derivatives has been achieved as SDS concentration above 15 mmol L^{-1}; moreover, all the seven derivatives were separated on baseline. Finally, 15 mmol L^{-1} SDS was chosen to use.

With the increase of applied voltage, the separation was improved very slightly, and the shape of peaks became bad when the separation voltage increased above 22.5 kV. Then 22.5 kV was chosen as the best separation voltage.

Using the optimized conditions, the seven phytohormones derivatives were successfully separated in 20 min and the typical electropherogram was shown in Fig. 3.

3.2. Optimization of derivatization conditions

Although the derivitization chemistry of APF with fatty acids has been investigated in our previous work [25], the derivitization conditions of APF with the seven phytohormones have been optimized in order to achieve the best derivatization yield.

Since the coexistence of APF and the condensing reagent, EDC may cause the formation of unwanted products owing to the carboxylic group APF molecules have, the concentrations of APF and EDC are the paramount factors for the derivatization efficiency and should be manipulated harmoniously in the derivatization procedure. In order to drive the reaction to completion, the use of excess APF was necessary. The effect of APF concentration on the derivatization was investigated within the range of 6×10^{-5} to 9.5×10^{-5} mol L^{-1} (Fig. 4-I). When APF concentration exceeded 9.0×10^{-5} mol L^{-1}, the peak areas of derivatives reached the maximum and remained stable. With further increase of APF concentration, the peak areas of derivatives did not change any

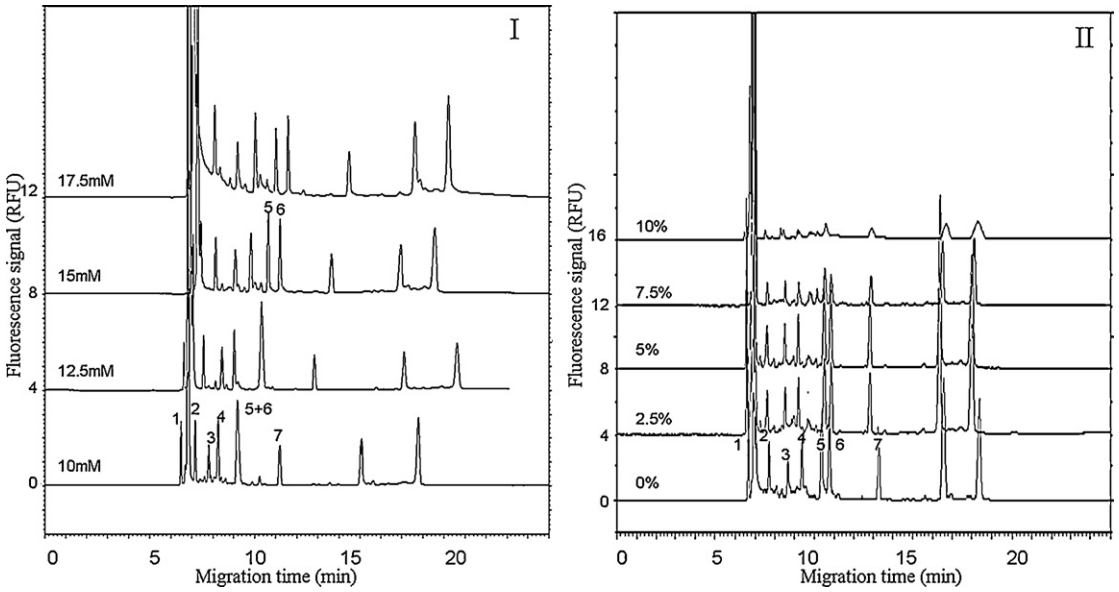

Fig. 2. (I) Electropherogram obtained from MECC mode in different concentration of SDS and (II) the effect of water content on the derivatization yields. Running buffer: 25 mmol L^{-1} borate buffer (pH 9.2); capillary: 65 cm (50 cm effective length) × 75 μm i.d.; injection: 5 s at 0.5 psi; separation voltage: 22.5 kV; temperature: 25 °C. Peaks: (1) GA3; (2) IAA; (3) ABA; (4) JA: (5) IBA; (6) NAA; (7) 2, 4-D.

more. Thus, 9.0×10^{-5} mol L^{-1} of APF concentrations was used in the following experiments.

The investigation of EDC from 3×10^{-3} to 5.5×10^{-3} mol L^{-1} indicated that the maximum peak areas appeared at EDC concentration of 4.5×10^{-3} mol L^{-1} and they declined when EDC concentration was larger than 5.0×10^{-3} mol L^{-1} (Fig. 4-II). Finally, the amount 4.5×10^{-3} mol L^{-1} of EDC was thus appropriate.

Reaction temperature can also affect the derivatization reaction significantly, and the temperature influence on the peak areas was studied from 40 to 75 °C. As shown in Fig. 4-III, the peak areas of the derivatives varied with the reaction temperature obviously. For the higher yields of all the derivatives simultaneously, 60 °C was selected for use. Additionally, the study of derivatization time revealed that the peak areas reached their plateaus from 60 min (Fig. 4-IV). Consequently, the derivatization should proceed at 60 °C for 60 min.

As a condensing reaction, the reaction of APF with the phytohormones releases water molecules, and water in plant samples might affect the derivatization of plant hormones and the accuracy of sample analysis. Accordingly, water content from 2.5% to 10% (v/v) was tested in the derivatization reaction. As showed in Fig. 2-II, under the optimized derivatization conditions, water content less than 5% has no significant effect on the derivatization yields in terms of the detection responses. In our sample preparation, the phytohormones in 0.5 g banana sample were extracted with 10 mL acetonitrile and thus water introduced from banana is much less than 5% apparently, which would not result in the reaction yield lost in the following derivatization.

3.3. Interference

APF may react with carboxylic group in amino acids and carboxylic acids under the chosen condition and finally bring about the interference for the determination of phytohormones. Therefore, the interference from C1 to C18 fatty acids, common amino acids has been evaluated thoroughly. Under the chosen separation conditions, the peaks of labeled carboxylic acid migrated behind of the peak of 2, 4-D. Since the extraction solvent was 10 mL acetonitrile which is a poor solvent for amino acids, and the water in 0.5 g banana samples was much less than 0.5 mL, amino acids may

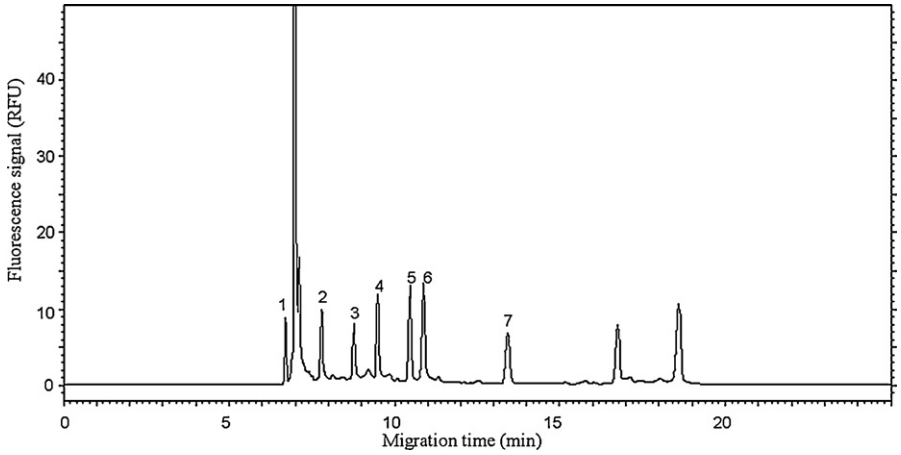

Fig. 3. Electropherogram of 1×10^{-7} mol L^{-1} standard phytohormones. Running buffer: 25 mmol L^{-1} borate buffer (pH 9.2), 15 mM SDS and 12% ethanol (v/v); capillary: 65 cm (50 cm effective length) × 75 μm i.d.; injection: 5 s at 0.5 psi; separation voltage: 22.5 kV; temperature: 25 °C. Peaks: (1) GA_3; (2) IAA; (3) ABA; (4) JA: (5) IBA; (6) NAA; (7) 2, 4-D.

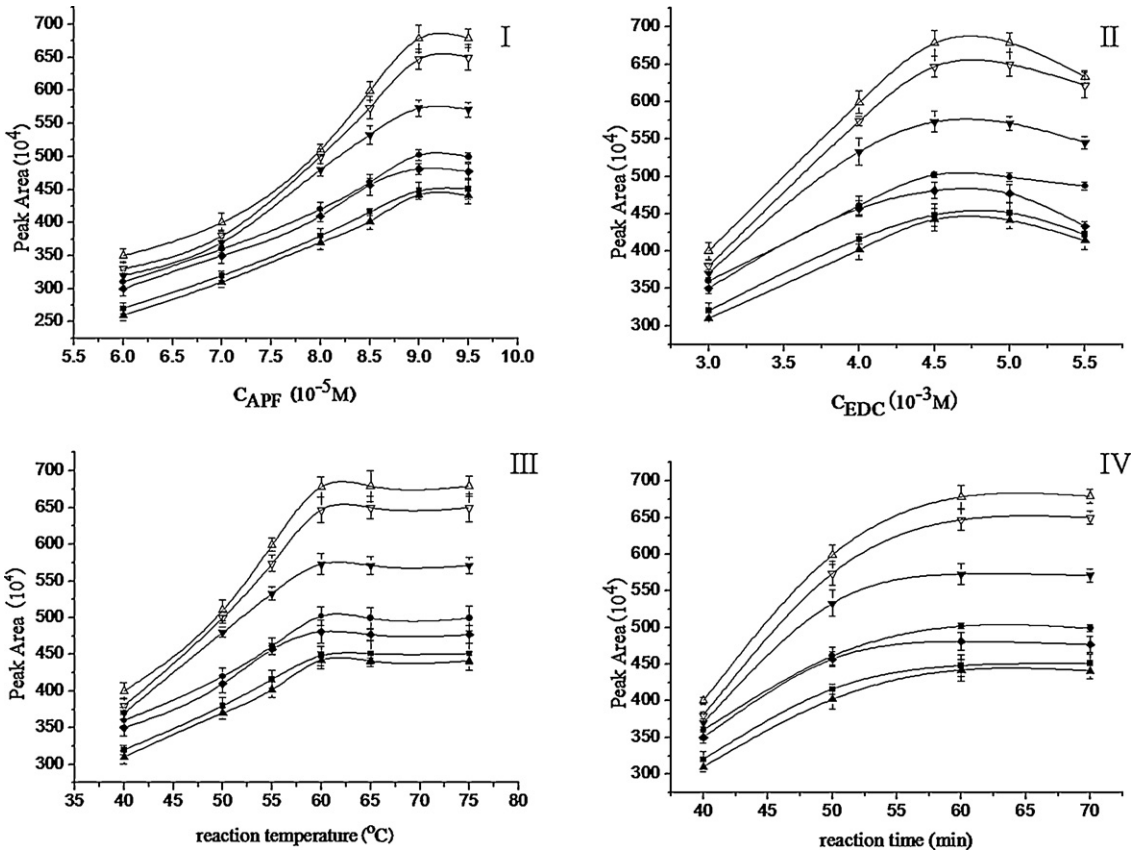

Fig. 4. Effects of (I) APF concentration, (II) EDC concentration, (III) reaction temperature and (IV) reaction time on the peak areas of APF derivatives. Phytohormones concentration: 1×10^{-7} mol L^{-1}. Symbol: (■) GA_3; (●) IAA; (♦) ABA; (▼) JA: (△) IBA; (▽) NAA; (▲) 2, 4-D.

not be extracted well in the supernatant. To assess the interference from amino acids, 16 common amino acids (1×10^{-3} mol L^{-1}, 1 μL, water solution) have been spiked in crude extracts of banana (200 μL). The electropherograms obtained were almost same for the samples with and without the spiking of amino acids.

3.4. Analytical calibration

By analyzing a series of the mixtures containing seven standard phytohormones at different concentrations (1×10^{-8} to 1×10^{-6} mol L^{-1}), the linear calibration ranges, regression equations, and limits of detection of them were calculated. The results are listed in Table 1. The correlation coefficients are from 0.9992 to 0.9998, and the RSDs are from 1.4% to 3.8% for within-day determination ($n = 6$) and from 2.4% to 3.6% for between-day determination ($n = 6$). The high reproducibility of the method indicated that CE–LIF based on APF derivatization was reliable for analyzing phytohormones. The limits of detection (S/N = 3) for the labeled phytohormones ranges from 1.6 nmol L^{-1} for ABA to 6.7 nmol L^{-1} for GA_3, and as the resulting solution of derivatization procedure was diluted five times before injection, the derivatization limits were about 20 nmol L^{-1}, which were sufficiently sensitive for the determination of trace amount phytohormones in plant samples.

3.5. Sample analysis

The proposed method has been applied to the determination of phytohormones in banana samples. The supernatant of the sample extracts were derivative and analyzed directly.

The electropherograms of the banana samples are given in Fig. 5. The phytohormone derivatives peaks were well separated from other interference peaks and identified by spiking standard. The analytical results of phytohormones in crude extracts of banana are summarized in Table 2. The recoveries are from 90.7 to 106.1% and RSDs vary from 1.8% to 4.6%. GA_3 and IAA were detected in the banana samples, and the levels of GA_3 and IAA decreased in the sample #2, which matches the common phenomenon of fruit ripening [28,29]. However, ABA and JA were not observed using the

Table 1
Liner calibration ranges, regression equations, and detection limits.

APF phytohormone derivative	Calibration range (μM)	R^2	RSD (%) $n = 6$, within-day	RSD (%) $n = 6$, between-day	Limits of detection[a] (nM)
GA3	0.02–1	0.995	2.6	3.2	6.7
IAA	0.02–1	0.993	2.7	2.4	4.8
ABA	0.01–1	0.997	3.4	2.0	1.6
JA	0.01–1	0.998	2.8	3.2	4.0
IBA	0.01–1	0.992	3.4	3.6	4.5
NAA	0.02–1	0.993	3.8	3.2	5.3
2,4-D	0.01–1	0.998	1.4	2.1	3.6

[a] S/N = 3.

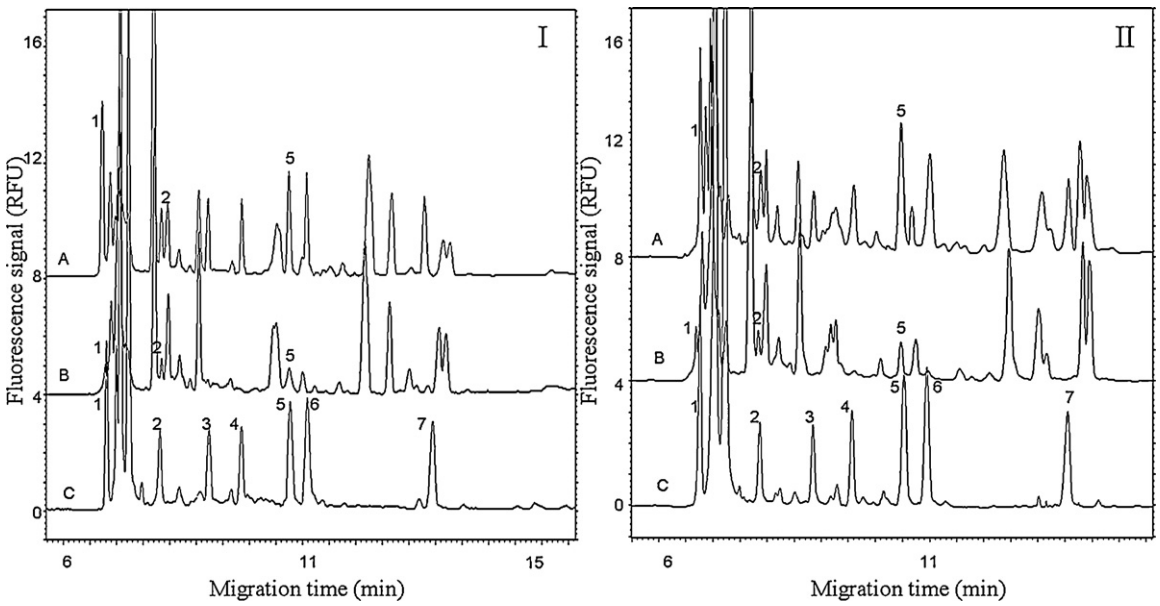

Fig. 5. Electropherogram obtained from banana sample: (I) banana sample #1; (II) banana sample #2. Running buffer: 25 mmol L^{-1} borate buffer (pH 9.2), 15 mM SDS and 12% ethanol (v/v); capillary: 65 cm (50 cm effective length) × 75 μm i.d.; injection: 5 s at 0.5 psi; separation voltage: 22.5 kV; temperature: 25 °C. A: sample spiked standard; B: sample; C: standard. Peaks: (1) GA_3; (2) IAA; (3) ABA; (4) JA: (5) IBA; (6) NAA; (7) 2, 4-D.

Table 2
Analytical results of real sample.

Analyte	Sample #1 (green) (ng/g)				Sample #2 (yellow) (ng/g)			
	Add	Found	RSD	Recovery	Add	Found	RSD	Recovery
GA3	0	311.7	3.3		0	249.3	2.8	
	1737	1995.0	3.7	97.2%	1737	1953.5	2.4	98.3%
IAA	0	119.1	4.6		0	99.6	3.7	
	525.6	665.3	3.2	106.1%	525.6	616.7	2.9	97.9%
ABA	0	N.D.			0	N.D.		
	792.9	782.3	2.6	98.7%	792.9	755.9	3.3	95.5%
JA	0	N.D.			0	N.D.		
	630.9	655.8	1.8	104%	630.9	626.4	1.8	99.3
IBA	0	280.1	2.6		0	101.5		
	609.6	833.1	1.9	90.7	609.6	703.0	2.6	98.7
NAA	0	N.D.			0	N.D.		
	558.6	569.8	3.1	102	558.6	536.2	3.6	96
2,4-D	0	N.D.			0	N.D.		
	663	604.9	2.7	96.7	663	667.4	2.4	100.6

Table 3
Comparison of the proposed method with former reports.

Analytical method	Sample preparation	Phytohormones	Internal standard	Detection limit	Cost	Ref
HPLC-tandem MS	Multi-extraction and SPE	Zeatin, GA, IAA, IBA, NAA, 2,4-D, ABA, BA	None	1.70–9.60 μM	High	[30]
HPLC-tandem MS	Crude extracts	Zeatin, GA, IAA, BA, ABA, IBA, JA, etc.	Isotopes	6×10^{-15} M to 6×10^{-13} M	High	[18]
GC-tandem MS	Multi-extraction and SPME	IAA, JA, SA, ABA, OPDA	Isotopes	10 nM–0.2 μM	High	[31]
CE–FD	Multi-extraction	IBA, IAA, IAAsp	None	15–28 nM	Low	[32]
CE–LIF	Crude extracts	ABA	None	1.1 nM	Low	[22]
CE–LIF	Crude extracts	GA3, IAA, IBA, ABA, NAA, IBA, 2,4-D	None	1.6–6.7 nM	Low	This work

proposed method. Besides, IBA at relatively high level was found, although it could not be concluded that IBA was applied as plant growth regulator.

4. Conclusions

A simple and rapid CE–LIF method for the simultaneous analysis of various phytohormones, such as GA_3, IAA, ABA, JA, IBA, NAA and 2, 4-D, has been developed. The strategy of APF-labeling coupled to CE–LIF allows rather high sensitivity and selectivity. Under the optimum derivatization and separation conditions, the limit of detection of ABA can reach 1.6 nmol L^{-1}, which is comparable to or better than those of phytohormone determination using other analytical methods (Table 3). Meanwhile, the crude extract from small portion of plant materials (0.5 g) can be directly analyzed without further treatment and the interference from the coexisting pigments and other organic substances are excluded.

Acknowledgments

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