Food Chemistry 170 (2015) 123–130

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

A simple and sensitive HPLC method based on pre-column fluorescence labelling for multiple classes of plant growth regulator determination in food samples

Guoliang Li [a,c,*], Shucheng Liu [c], Zhiwei Sun [a], Lian Xia [a], Guang Chen [a], Jinmao You [a,b,*]

[a] Key Laboratory of Life-Organic Analysis of Shandong Province, Qufu Normal University, Qufu, People's Republic of China
[b] Key Laboratory of Tibetan Medicine Research, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, People's Republic of China
[c] College of Food Science and Technology, Guangdong Ocean University, Guangdong Provincial Key Laboratory of Aquatic Product Processing and Safety, Key Laboratory of Advanced Processing of Aquatic Products of Guangdong Higher Education Institution, Zhanjiang, People's Republic of China

ARTICLE INFO

Article history:
Received 17 December 2013
Received in revised form 23 June 2014
Accepted 30 July 2014
Available online 17 August 2014

Keywords:
Plant growth regulators
Pre-column fluorescence labelling
HPLC
Food samples

ABSTRACT

The determination of trace plant growth regulator (PGR) has received more and more attentions in the field of phytophysiology and food safety. But the simple and sensitive method for simultaneously analysing multiple classes of PGR remains poorly investigated. In this study, a new pre-column fluorescence labelling method using 2-(11H-benzo[a]carbazol-11-yl)-ethyl-4-methylbenzenesulfonate (BCETS) as the labelling reagent has been developed for simultaneous determination of seven PGRs (i.e., indole-3-acetic acid, 3-indolybutyric acid, 3-indolepropionic acid, jasmonic acid, gibberellin A_3, 1-naphthylacetic acid and 2-naphthaleneacetic acid) by HPLC with fluorescent detection (FLD). The proposed method offered the LOD of 0.34–0.73 ng/mL for seven PGRs, which were significantly lower than the reported methods. The crude extract without complex pre-treatments and purification was directly labelled by BCETS and analysed by HPLC-FLD, which facilitates the high-throughput sample screening. This method was proven to be inexpensive, simple, selective, sensitive, accurate and reliable for trace PGR determination.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Plant growth regulators (PGRs), either produced naturally by the plant or synthesized by human, are small organic molecules that alter the growth and development of plants (Francis & Sorrell, 2001; Santner, Calderon-Villalobos, & Estelle, 2009; Santner & Estelle, 2009). For example, gibberellic acids are present in higher plants acting as endogenous growth regulator, and their main physiological effects include the induction of germination and flowering, seedless development of some fruit in the absence of fertilisation and delay of senescence in leaves and citrus fruits (Davies, 1995). Indole-3-acetic acid (IAA) stimulates growing processes, and abscisic acid (ABA) controls plant senescence (Davies, 1995; Santner et al., 2009). 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 (Wasternack & Parthier, 1997; Wasternack et al., 2006). Furthermore, JA can affect the skin colour of apple and the content of flavonoid in apple, which has been applied to increase the red blush of apple. Recently, the use of PGRs is becoming popular to enhance plant growth and crop yield (Cho et al., 2013; Taglienti et al., 2011). Many PGRs have been extensively applied in many countries such as Australia, Japan, China and India, which has led to more concerns about their toxicity and residues in edible plants (Cho et al., 2013; Xue et al., 2011). Legislative bodies have indicated maximum residue limits for health protection, and maintaining the PGR residue concentration as low as possible in commodities is one of the most important quality criteria in market monitoring and international trade (Xue et al., 2011). However, to the best of our knowledge, the study for simultaneously analysing multiple classes of PGRs in plants remains poorly investigated, which signifies a simple method with highly sensitivity and selectivity is of great significance for the field of psychophysiology and food safety.

Unfortunately, accurate analysis of PGRs becomes a very challenging task. For example, PGRs in plants are present at very low concentrations against a background of a wide range of abundant primary and secondary metabolites. Thus, the analytical methods to quantitate these compounds simultaneously must be extremely

* Corresponding authors. Address: Key Laboratory of Life-Organic Analysis of Shandong Province, Qufu Normal University, Qufu, People's Republic of China. Tel.: +86 537 4456305.
E-mail addresses: 61254368@163.com (G. Li), jmyou6304@163.com (J. You).

http://dx.doi.org/10.1016/j.foodchem.2014.07.146
0308-8146/© 2014 Elsevier Ltd. All rights reserved.

selective and sensitive. Many PGRs with carboxylic group possess strong polarity, which causes them to be retained weakly in reversed-phase LC systems and makes their separation in traditional analytical methods more difficult (Xue et al., 2011). Furthermore, some PGRs such as jasmonates, gibberellins and abscisic acid have little ultraviolet (UV) absorption, no fluorescence and no distinguishing chemical characteristics, so accurate determination using absorptiometry is fairly difficult. Different types of methods have been described to estimate PGRs in plants such as gas chromatography–mass spectrometry (GC–MS), high-performance liquid chromatography–mass spectrometry (HPLC–MS) and capillary electrophoresis–mass spectrometry (CE–MS) (Chen, Guo, Zhang, & Wang, 2011; Du, Ruan, & Liu, 2012; Durgbanshi et al., 2005; Fu, Sun, Wang, Chu, & Yan, 2011; Hou, Zhu, Ding, & Lv, 2008; Prasad et al., 2010). Each of these methods above has its own characteristics, but they have some limitations in PGR determination such as limited classes of plant hormones, low selectivity and sensitivity, or poor applicability. For example, GC–MS needs a complicated and intensive purification protocol, which was time-consuming and tedious. Some thermally labile PGRs are likely to break down at the high temperature of the GC injector and column, which limits the range of plant hormones fit for GC analysis (Han et al., 2012). HPLC–MS method is another choice for PGR determination. But ESI-MS/MS in the negative-ion mode sometimes does not demonstrate the required sensitivity for the trace analysis of PGRs. Because the best chromatographic resolution with reversed-phase HPLC is achieved at an acidic pH, in which condition the ionization of the carboxyl groups is suppressed. Moreover, HPLC–MS methods often require high resolution mass spectrometry to ensure the high detection sensitivity, not easily available in common analytical laboratories. Besides, some bio-analytical means such as radio immunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) were also reported. However, these methods used expensive instruments or have been focused on analysis of a single compound or had insufficient sensitivity (Du et al., 2012; Fu et al., 2011). Chemical derivatization is a feasible way to improve the detection sensitivity and selectivity of HPLC method. PGRs can be labelled selectively by fluorescence labelling reagent and detected by FLD at specific excitation and emission wavelengths. Thus, the labelling procure could significantly decrease the interference of primary and secondary metabolites in real samples and improve the detection selectivity (Yu et al., 2010). However, analytical method for PGR determination based on pre-column fluorescent labelling is rarely reported. Although some labelling reagents such as dansyl hydrazine, 9-anthryldiazomethane, 8-aminopyrene-1,3,6-trisulfonate, and 3-(2-furoyl)quinoline-2-carboxaldehyde, have been developed for the determination of phytohormones with a carboxyl group, these reagents show many shortages such as low detection sensitivity, poor stability, low stability of the derivatives, tedious labelling time, fussy analytical procedure and so on (Chen et al., 2010; Du et al., 2012; Xiong, Rao, Guo, Wang, & Zhang, 2012). Recently, a new fluorescent labelling reagent 2-(11H-benzo[a]carbazol-11-yl)-ethyl-4-methylbenzenesulfonate (BCETS) has been designed successfully in our research group (Li et al., 2011). This labelling reagent possessed stronger photoluminescence property, ensuring the high sensitive detection. In this study, BCETS was first employed for simultaneous determination of seven PGRs (i.e., indole-3-acetic acid, 3-indolybutyric acid, 3-indolepropionic acid, jasmonic acid, gibberellin A_3, 1-naphthylacetic acid and 2-naphthaleneacetic acid) by HPLC-FLD. Moreover, the extraction solvents and fluorescence labelling conditions for PGRs were optimized in order to ensure the sufficient extraction and labelling. The proposed method was validated and applied to real sample determination, which was proven to be efficient, selective, sensitive and accurate for multiple PGR analysis in foodstuffs.

Furthermore, the sample pre-treatment is a headache in PGR analysis especially for the analysis targeting multiclass phytohormones. Solid phase extraction (SPE) and liquid–liquid phase microextraction (LLME) are often used for sample purification, but these methods often required tedious purification procedure (Du et al., 2012; Fu et al., 2011). In the present study, crude extract was labelled by BCETS and directly analysed by HPLC-FLD without complex pre-treatments, which is a key factor in high-throughput sample screening.

2. Materials and methods

2.1. Reagents and standards

Standards including indole-3-acetic acid (IAA), 3-indolybutyric acid (IBA), 3-indolepropionic acid (IPA), jasmonic acid (JA), gibberellin A_3 (GA), 1-naphthylacetic acid (1-NAA) and 2-naphthaleneacetic acid (2-NAA) were purchased from Sigma–Aldrich (Sigma–Aldrich Company, USA) and the chemical structures are shown in Supplementary Fig. S1. Stock solutions (1 mg/mL of each analyte) were prepared by dissolving PGRs in N,N-Dimethylformamide (DMF), respectively. All stock solutions were stored at 4 °C in the refrigerator. Working standard solutions were obtained by stepwise dilution of their stock standard solutions with DMF. High purity water purified with a Milli-Q water purification system was used throughout the experiment. HPLC grade acetonitrile (ACN) was purchased from Yucheng Chemical Reagent Co. (Yucheng, Shandong Province, China). Other chemicals were analytical grade from Jining Chemical Reagent (Jining, Shandong Province, China).

2.2. Instrument and conditions

An Agilent 1100 HPLC system was employed for HPLC analysis, which was coupled on-line to a fluorescence detector (FLD) and an ion-trap mass spectrometer (Agilent Corp., Waldbronn, Germany) equipped with an atmospheric pressure chemical ionization (APCI) source. HPLC separation was carried out on a reversed-phase Hypersil BDS C_{18} column (200 mm × 4.6 mm, 5 μm) with a gradient elution. The mobile phase A was 20% ACN containing 0.1% ammonium formate and B was 100% ACN. The column temperature was set at 30 °C. The solvent flow rate was 1 mL/min and the detection wavelength was set as λex/λem = 279/380 nm. The gradient elution program was as follows: 0 min = 55% B, 8 min = 60% B, 13 min = 80% B, and 20 min = 95% B. The auto MS operation parameters were as follows: APCI in positive ion detection mode; nebulizer pressure 60 psi; dry gas temperature, 350 °C; dry gas flow, 5.0 L/min. APCI Vap temperature 350 °C; corona current 4000 nA; capillary voltage 3500 V.

2.3. Sample preparation

All plant samples including peach, bananas, apple, potato and tomato were randomly collected from local markets in Qufu, Shandong province. All samples were cut into pieces, and then homogenised with high speed homogenizer. The homogenised plant samples (1 g) were further homogenised for 2 min with cold ACN containing 1% formic acid (3 mL), then extracted twice for 10 min at 4 °C by ultrasonic. The mixture was centrifuged (4500 rpm, 4 °C) for 10 min, and the supernatant were combined. A 3 mL of supernatant were evaporated to dryness by a gentle nitrogen stream, and re-dissolved by 0.5 mL DMF. The solution was filtered with a 0.45 μm filter and then stored at 4 °C for use.

2.4. Optimization of derivatization

The derivatization procedure for BCETS with jasmonic acid is shown in Fig. 1A. According to our previous study (Li et al.,

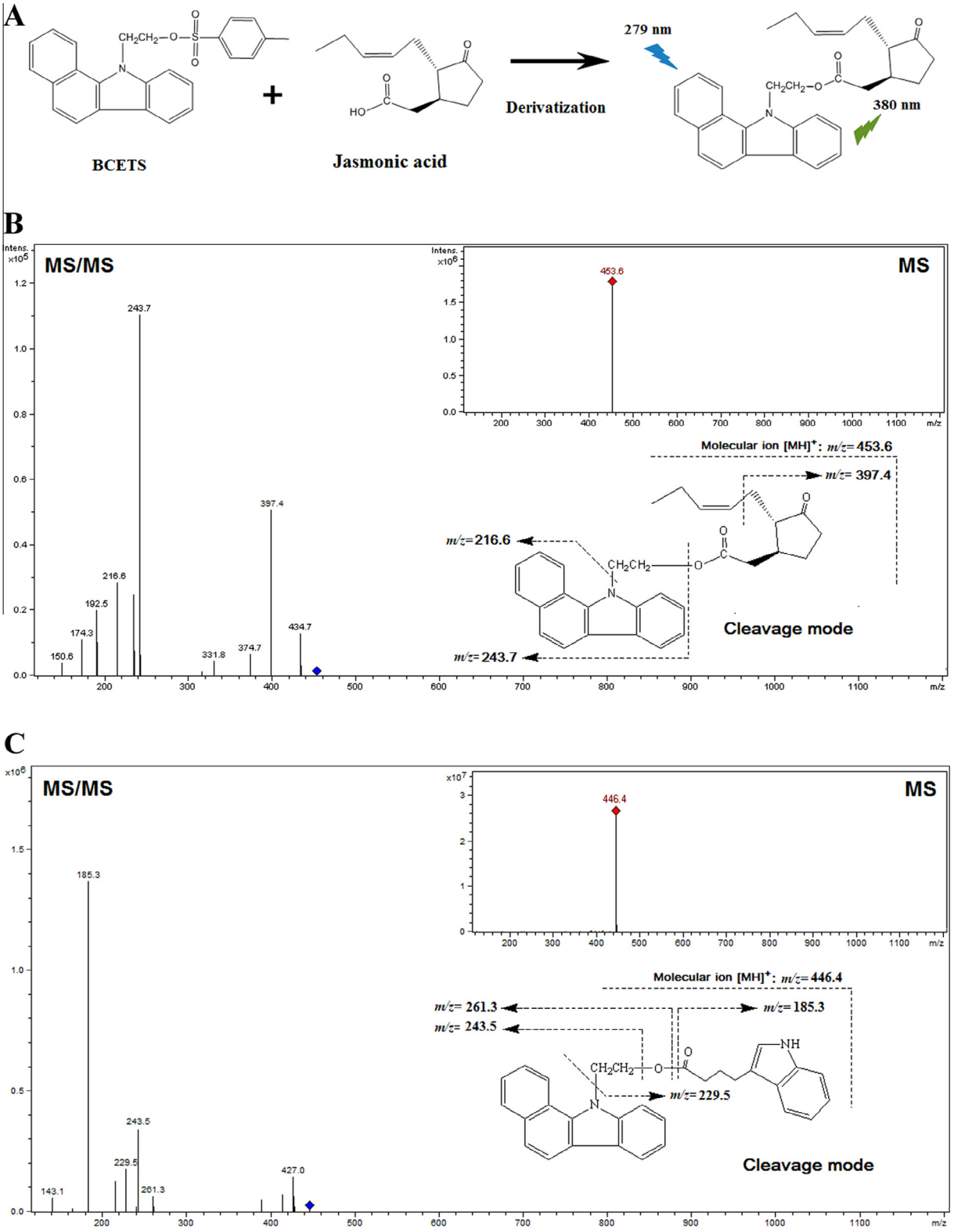

Fig. 1. The representative labelling scheme for BCETS with jasmonic acid under the optimum conditions (A), and the representative MS data (MS and MS/MS) and cleavage mode for BCETS–JA derivative (B) and BCETS–IBA (C) derivative.

2011), the main parameters affecting the derivatization efficiency were the co-solvents, derivatization time, temperature and concentration of BCETS. These conditions were optimized separately, and JA, GA, 1-NAA and IAA were used as the tested compounds. After the reaction was completed, the mixture was cooled to room temperature, and 200 μL volume of pure acetonitrile was added to dilute the derivatization solution, and the diluted solution (10 μL) was injected directly onto the chromatograph.

2.5. Method validation

The linearity, repeatability, accuracy, precision, sensitivity, recovery, and limits of detection (LOD) were validated according to United States Food and Drug Administration (FDA) guidelines (Nielsen, 2010) and several reported methods (Han et al., 2012; Li et al., 2011). For linearity evaluation, standard solutions at a series of concentrations (0.0125–6.35 μmol/L) were prepared and injected into the HPLC system. Calibration curves were constructed by linear regression of the peak area (Y) versus the concentration (X). The LOD were calculated at the signal-to-noise (S/N) ratio of 3. The method repeatability was investigated by measuring the relative standard deviations (R.S.D.%) for peak area and retention time. The accuracy of the analytical method was determined by spiking a known amount of standards into real samples and analysing the percentage recovery. The precision was expressed as the percentage relative standard deviation (R.S.D.%).

3. Results and discussion

3.1. Selection of extraction method

Sample preparation impacts nearly all the subsequent assay steps and is hence critical for the unequivocal identification, confirmation, and quantification of analytes, especially those present at trace or ultra-trace levels in complex matrices. Many common PGRs have a carboxyl group in their structure, which are structurally and chemically diverse compounds. Therefore, it is pivotal to choose proper approaches for PGR extraction before their analysis. By referring to several reported methods for plant hormone extraction (Bai, Du, & Liu, 2010; Chen, Guo, et al., 2011; Shi et al., 2011), several extraction solvents have been tried in the present study such as (a) MeOH/H_2O/HCOOH (80/19/1, v/v/v), (b) ACN/H_2O/HCOOH (80/19/1, v/v/v), (c) MeOH/HCOOH (99/1, v/v) and (d) ACN/HCOOH (99/1, v/v). Apple samples were spiked with the intermediate levels of each standard solution. The samples were macerated with 20 mL of the candidate solutions and pretreated as described in Sample Preparation Section. Results in Fig. 2 indicated each solvent has its merit for the extraction of several targeted compound. For example, solvent (a) yielded the recoveries of 103% and 96% for IAA and JA, respectively, but not good enough recoveries for 1-NAA (70%) and GA (77%). Solvent (b) and Solvent (c) showed the recoveries of 80–105%, and 83–110% for all compounds, respectively. The best results were given when Solvent (d) was used as the extractant solvent, and recoveries higher than 94% were obtained for all the compounds. Finally, Solvent (d): ACN/HCOOH (99/1, v/v) was used as the optimum solvent.

3.2. Optimization of derivatization conditions

The derivatization conditions of BCETS with the seven PGRs have been optimized in order to achieve the best derivatization yield. The co-solvents for derivatization including DMF, ACN, ethyl acetate and DMSO were investigated. DMF gave the most intense fluorescence responses and can avoid the problem of precipitation of PGR derivatives. Thus DMF was chosen as the co-solvent. The results also indicated that the added K_2CO_3 amount of 20 mg was the best basic catalyst and provided the highest detection responses. Temperature influence on derivatization reaction was studied from 60 to 100 °C. By fixing the concentration of the reactants and reaction time, the peak area of the analyte reached its apex when the temperature was 90 °C. Higher temperature could result in the decrease of the derivatization yield. Therefore, 90 °C was chosen as the derivatization reaction temperature. The influence of BCETS amount (the molar ratio of BCETS to PGRs) on the derivatization was investigated within the range of 2–9. When the molar ratio of BCETS to PGRs is 5, the peak area of the derivatives reached a maximum, and was chosen as the optimal concentration of BCETS. The effect of the reaction time from 10 to 40 min at 90 °C has also been examined. In the tested range, the peak areas of all PGR derivatives increased and kept constant after 20 min. Thus, the derivatization was carried out for 20 min.

Finally, the optimum labelling conditions were obtained and the labelling procedure was as follow: (1) To a 1 mL vial, 20 μL BCETS solution, 20 mg K_2CO_3, 20 μL standards mixture (3×10^{-4} M, or 200 μL sample solution) and 200 μL DMF was

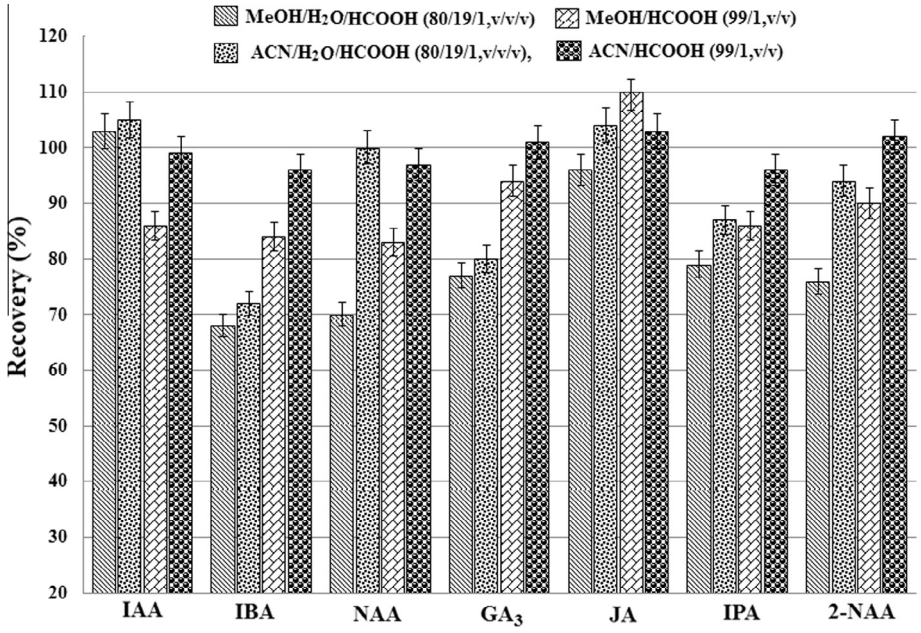

Fig. 2. The recoveries of the selected solvents (MeOH/H_2O/HCOOH (80/19/1, v/v/v), ACN/H_2O/HCOOH (80/19/1, v/v/v), MeOH/HCOOH (99/1, v/v) and ACN/HCOOH (99/1, v/v)).

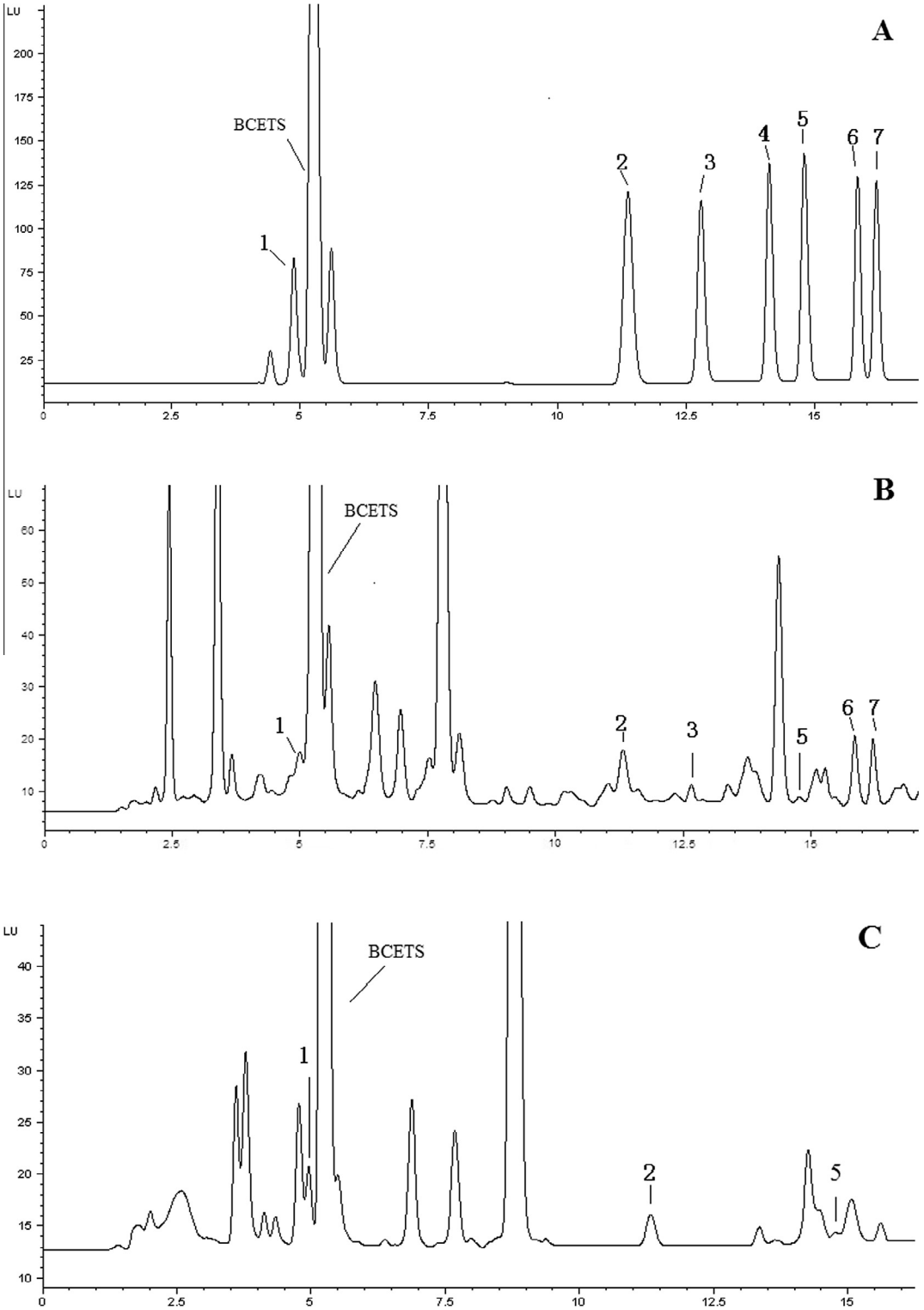

Fig. 3. The representative chromatograms for seven standards (A), apple sample (B) and peach sample (C). Peak label: (1) gibberellin A_3; (2) indole-3-acetic acid; (3) 3-indolepropionic acid; (4) 3-indolybutyric acid; (5) jasmonic acid; (6) 1-naphthylacetic acid and (7) 2-naphthaleneacetic acid.

successively added; (2) The vial was sealed and allowed to react in a water bath at 90 °C with shaking in 5 min intervals for 20 min.

3.3. HPLC separation and MS identification

The proportion of acetonitrile in the mobile phase was investigated. The retention time of PGR derivatives gradually reduced with increasing acetonitrile percentage. But the excessive proportion of acetonitrile was adverse to the complete separation of seven PGR derivatives in real samples, because the crude extracts of plant samples were very complicated without purification pre-treatment. The optimum HPLC conditions were described in Section 2.2. Under these conditions, seven targeted PGRs can achieve baseline separation with good peak shape in 17 min. The typical chromatogram of seven PGR derivatives is given in Fig. 3A. Other acidic compounds might coexist in plant samples,

Table 1
Linear regression equation, correlation coefficients, LOD, reproducibility of retention time and peak area, accuracy and intra- and inter-day precision.

Analytes	Linearity		LOD[b] (nmol/L)	LOD[b] (ng/mL)	Repeatability R.S.D. (%) (n = 6)		Precision R.S.D. (%) (n = 6)	
	$Y = AX + B$[a]	R^2			Retention time	Peak area	Intra-day	Inter-day
GA	$Y = 27.43X + 2.36$	0.9997	2.1	0.73	0.03	1.65	2.15	4.44
IAA	$Y = 46.30X + 1.74$	0.9995	2.4	0.42	0.04	1.43	4.00	5.73
IPA	$Y = 32.27X + 3.43$	0.9996	2.6	0.49	0.01	1.22	2.10	5.21
IBA	$Y = 33.08X + 0.82$	0.9996	2.3	0.47	0.02	1.53	1.72	4.20
JA	$Y = 40.95X + 1.67$	0.9998	1.7	0.36	0.01	1.60	3.30	5.10
1-NAA	$Y = 30.22X + 0.67$	0.9999	2.2	0.41	0.04	1.84	3.80	4.73
2-NAA	$Y = 27.99X + 2.17$	0.9997	1.8	0.34	0.02	2.00	3.05	5.60

[a] Y, peak area; X, injected amount of each triterpenic acid (ng).
[b] S/N = 3, per 10 μL injection volume.

which might be labelled by BCETS. In this study, the chromatogram peak was doubly confirmed by comparing the retention time of standard compound and online mass spectrometry identification (APCI/MS in positive mode). The MS data indicated that PGR derivatives exhibited intense quasi-molecular ion peak of $[M+H]^+$, and the MS/MS data showed that PGR derivatives gave the main fragment ions like m/z 185, m/z 216 and m/z 243, which are formed by cleavage of ester bond, N–CH_2 bond and CH_2–O bond (Fig. 1). The MS, MS/MS and cleavage mode of BCETS–JA and BCETS–IBA are presented in Fig. 1. BCETS–JA produced an intense molecular ion peak at m/z 453.6 and the specific fragment ions at m/z 397.4, m/z 243.7, and m/z 216.6 (Fig. 1B). BCETS–IBA produced an intense molecular ion peak at m/z 446.4 and the specific fragment ions at m/z 185.3, m/z 229.5, m/z 243.5 and m/z 261.3 (Fig. 1C).

3.4. Method validation

The linearity of the chromatographic responses versus concentrations was studied. Goodness of fit was estimated by the correlation coefficient (R^2). As shown in Table 1, the adequate goodness of fit ranged from 0.9995 to 0.9999. Limit of detection (LOD) values were in the range of 1.7 (JA)–2.6 nM (IPA). These results indicate that the proposed HPLC method is ultrasensitive to quantify PGRs in plant samples. RSD values of retention time and peak area of seven compounds were less than 0.04% and 2.0%, respectively, which satisfied the criteria of quantitative analysis. The intra- and inter-day precisions (expressed in terms of % R.S.D.) were found to be in the range of 1.72–4.00% and 4.20–5.73%, respectively, which demonstrated the good precision of the proposed method. The accuracy of the analytical procedure was evaluated using the recovery test. This involved the addition of known quantities of standard reference compounds to the sample and analysed using the optimal conditions. The percentage of recovery obtained by comparing the results from the original samples and the fortified samples are reported in Table 3. The recovery rates obtained were in the range of 94–104%, this method can be considered to be accurate enough (Table 3). The validation data indicated that the proposed method provides good linearity, sensitivity, procedure accuracy, precision, as well as excellent suitability for the simultaneous analysis of seven PGRs in plant samples.

A comparison of the proposed method with the recently reported methods is provided in Table 2. The proposed method offered the LOD of 1.7–2.6 nM (or 0.34–0.73 ng/mL) for seven PGRs, which were significantly lower than the reported methods in Table 2. BCETS can perform the sufficient labelling of the analytes in as little as 20 min, which is more rapid than the reported reagents (1 h and 12 h) in Table 2. Furthermore, most of the methods need the tedious purifications (e.g., solid-phase microextraction (SPE), liquid–liquid extraction (LLE) and immunoextraction). In this method, the crude extract was labelled by BCETS and directly analysed by HPLC-FLD without complex pre-treatments, which is a key factor in high-throughput sample screening.

3.5. Sample analysis

The developed method was applied to the simultaneous determination of seven PGRs in several plant samples including peach, bananas, apple, potato and tomato. The representative

Table 2
The overall comparison of the new method with reported methods.

The reported methods for PGRs determination					
Analyte	Method	Reagent	Labelling condition	LOD (ng/mL or nM)	References
GA, IAA, JA, IBA, NAA, 2,4-D	CE-LIF	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide	60 °C, 60 min	3.6–6.7 nM	Chen, Guo, et al. (2011)
IBA, NAA, 2,4-D	HPLC-FLD	6-Oxy(acetylpiperazine) fluorescein	60 °C, 60 min	4.43–14.8 nM	Chen et al. (2010)
JA	HPLC-ECD	Dopamine	60 °C, 12 h	50 nM	Xie, Wang, and Chen (2013)
SA, IAA, ABA, JA	HF–LLLME-HPLC–UV[a]	–	–	0.9–4.6 ng	Wu and Hu (2009)
GA, IAA, ABA	SPE-LC–MS/MS[b]	–	–	2200 ng	Hou et al. (2008)
IAA, IBA	LLE-HPLC–UV[c]	–	–	3900–7900 ng	Zhang, Li, Hu, Li, and Chen (2010)
GA, JA, SA, IAA, IBA, ABA	CE–ESI–ToF-MS	–	–	0.34–4.59 ng	Chen, Guo, et al. (2011)
1-NAA, 2-NAA	MS-RTP[c]	–	–	11.5–15.6 ng	Murillo Pulgarín, García Bermejo, Sánchez-Ferrer Robles, and Becedas Rodríguez (2012)
GA, IAA, IPAI, BA, JA, 1-NAA, 2-NAA	HPLC-FLD	BCETS	90 °C, 20 min	0.34–0.73 ng/mL or 1.7–2.6 nM	This study

[a] Hollow fibre-based liquid–liquid–liquid microextraction (HF–LLLME).
[b] Solid-phase extraction.
[c] Micelle-stabilised room temperature phosphorescence (MS-RTP).

Table 3
Determination of seven plant growth regulator in plant samples ($n = 3$).

Food sample	(ng/g)	GA	IAA	IPA	IBA	JA	1-NAA	2-NAA
Potato	Added	0	0	0	0	0	0	0
	Found	26.7	0	6.8	0	3.51	0	0
	Added	85	95	15	10	15	145	140
	Found	109.1	90.8	20.9	10.2	19.2	137.3	138.1
	Recovery (%)	97.7	96.1	96.4	101.1	103.6	95	98.8
Bananas	Added	0	0	0	0	0	0	0
	Found	8.35	88.2	0	0	0	0	0
	Added	85	95	15	10	15	145	140
	Found	87.9	175.9	14.1	9.5	14.2	138.9	143.5
	Recovery (%)	94.2	96	94	95	94.6	95.8	102.5
Peach	Added	0	0	0	0	0	0	0
	Found	20.8	5.46	12.44	0	4.2	140.2	135.1
	Added	85	95	15	10	15	145	140
	Found	108.5	99.7	26.9	10.5	16.8	279.3	262.9
	Recovery (%)	102.3	99.2	98.0	105	97.5	97.9	95.6
Tomato	Added	0	0	0	0	0	0	0
	Found	18.9	15.6	10.33	0	0	0	0
	Added	85	95	15	10	15	145	140
	Found	99.2	115.4	25.0	9.6	14.7	139.1	145.5
	Recovery (%)	95.5	104.3	98.6	96.5	98.3	96	104
Apple	Added	0	0	0	0	0	0	0
	Found	79.4	91.2	0	0	10.43	37.56	0
	Added	85	95	15	10	15	145	140
	Found	157.7	184.0	14.5	10.1	24.9	180.2	141.5
	Recovery (%)	96	99	96.4	100.8	98.1	98.7	101.1

chromatograms for apple and peach are given in Fig. 3B and C, respectively. Each sample was determined in triplicate, and the peaks in chromatograms were identified by the retention time and online mass spectroscopy. As shown in Table 3, the concentration of the targeted PGRs varied greatly among the different samples. GA was found in all samples with the content range of 8.35–79.4 ng/g. IPA was detected in tomato, potato and peach, and the contents were 10.33, 6.8 and 12.44 ng/g, respectively. IAA was determined in all samples except for potato sample, peach and apple represented the sample with the lowest and highest content of 5.46 and 91.2 ng/g, respectively. JA was identified in potato (3.51 ng/g), peach (4.2 ng/g) and apple (10.43 ng/g), and 1-NAA was only detected in apple (37.56 ng/g). However, 2-NAA was not observed in all five samples.

Shi et al. established a new HPLC-MS method for PGR determination (Shi et al., 2011), peach and apple were analysed, GA was only determined in peach sample (16.5–20 ng/g), which was closed to the results obtained in the present study. Murillo Pulgarín developed a novel method for 1-NAA and 2-NOA analysis based on room temperature phosphorescence (Murillo Pulgarín, García Bermejo, Sánchez-Ferrer Robles, & Becedas Rodríguez, 2012). All samples were free of NAA and NOA contamination. In our study, 1-NAA was detected in peach and apple sample, which indicated they had been subjected to chemical phytosanitary treatment.

The crude extract from small portion of plant material can be directly analysed without further treatment, and the interference from the coexisting pigments can be decreased significantly by the selective derivatization. From the above research results, the proposed method had been elucidated to be simple, inexpensive, selective, sensitive, accurate and reliable for trace PGR determination in plant samples.

4. Conclusion

In this study, a new pre-column fluorescence labelling method using BCETS as the labelling reagent has been developed for multiple classes of PGR analysis by HPLC-FLD. This method has been proven to be simple, inexpensive, selective, sensitive, accurate and reliable for trace PGR determination. Furthermore, this developed method should have powerful potential in the analysis of exogenous and endogenous PGR with a carboxyl group from many other complex samples.

Acknowledgements

This work was supported by The National Natural Science Foundation of China (Nos. 31301595, 21275089 and 21305076), Guangdong Provincial Key Laboratory of Aquatic Product Processing and Safety (GDPKLAPPS1401), The Scientific Research Fund of Qufu Normal University (xkj201302) and Ph.D. research start-up funds of Qufu Normal University (BSQD2012017, BSQD20110119).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.07.146.

References

Bai, Y., Du, F., & Liu, H. (2010). Determination strategies of phytohormones: Recent advances. *Analytical Methods, 2*(12), 1867–1873.
Chen, H., Guo, X. F., Zhang, H. S., & Wang, H. (2011). Simultaneous determination of phytohormones containing carboxyl in crude extracts of fruit samples based on chemical derivatization by capillary electrophoresis with laser-induced fluorescence detection. *Journal of Chromatography B, 879*(20), 1802–1808.
Chen, H., Zhang, Z. X., Zhang, G. M., Guo, X. F., Zhang, H. S., & Wang, H. (2010). Liquid chromatographic determination of endogenous phytohormones in vegetable samples based on chemical derivatization with 6-oxy (acetylpiperazine) fluorescein. *Journal of Agricultural and Food Chemistry, 58*(8), 4560–4564.
Cho, S. K., Abd El-Aty, A., Park, K. H., Park, J. H., Assayed, M., Jeong, Y. M., et al. (2013). Simple multiresidue extraction method for the determination of fungicides and plant growth regulator in bean sprouts using low temperature partitioning and tandem mass spectrometry. *Food Chemistry, 136*, 1414–1420.
Davies, P. J. (1995). *Plant hormones: Physiology, biochemistry and molecular biology*. The Netherlands: Kluwer Academic Dordrecht.
Du, F., Ruan, G., & Liu, H. (2012). Analytical methods for tracing plant hormones. *Analytical and Bioanalytical Chemistry, 403*(1), 55–74.
Durgbanshi, A., Arbona, V., Pozo, O., Miersch, O., Sancho, J. V., & Gomez-Cadenas, A. (2005). Simultaneous determination of multiple phytohormones in plant

extracts by liquid chromatography–electrospray tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 53*(22), 8437–8442.
Francis, D., & Sorrell, D. A. (2001). The interface between the cell cycle and plant growth regulators: A mini review. *Plant Growth Regulation, 33*(1), 1–12.
Fu, J., Sun, X., Wang, J., Chu, J., & Yan, C. (2011). Progress in quantitative analysis of plant hormones. *Chinese Science Bulletin, 56*(4–5), 355–366.
Han, Z., Liu, G., Rao, Q., Bai, B., Zhao, Z., Liu, H., et al. (2012). A liquid chromatography tandem mass spectrometry method for simultaneous determination of acid/alkaline phytohormones in grapes. *Journal of Chromatography B, 881*, 83–89.
Hou, S., Zhu, J., Ding, M., & Lv, G. (2008). Simultaneous determination of gibberellic acid, indole-3-acetic acid and abscisic acid in wheat extracts by solid-phase extraction and liquid chromatography–electrospray tandem mass spectrometry. *Talanta, 76*(4), 798–802.
Li, G., You, J., Suo, Y., Song, C., Sun, Z., Xia, L., et al. (2011). A developed pre-column derivatization method for the determination of free fatty acids in edible oils by reversed-phase HPLC with fluorescence detection and its application to *Lycium barbarum* seed oil. *Food Chemistry, 125*(4), 1365–1372.
Murillo Pulgarín, J. A., García Bermejo, L. F., Sánchez-Ferrer Robles, I., & Becedas Rodríguez, S. (2012). Simultaneous determination of plant growth regulators 1-naphthylacetic acid and 2-naphthoxyacetic acid in fruit and vegetable samples by room temperature phosphorescence. *Phytochemical Analysis, 23*(3), 214–221.
Nielsen, S. S. (2010). *United States government regulations and international standards related to food analysis*. Springer.
Prasad, K., Das, A. K., Oza, M. D., Brahmbhatt, H., Siddhanta, A. K., Meena, R., et al. (2010). Detection and quantification of some plant growth regulators in a seaweed-based foliar spray employing a mass spectrometric technique sans chromatographic separation. *Journal of Agricultural and Food Chemistry, 58*(8), 4594–4601.
Santner, A., Calderon-Villalobos, L. I. A., & Estelle, M. (2009). Plant hormones are versatile chemical regulators of plant growth. *Nature Chemical Biology, 5*(5), 301–307.
Santner, A., & Estelle, M. (2009). Recent advances and emerging trends in plant hormone signalling. *Nature, 459*(7250), 1071–1078.
Shi, X., Jin, F., Huang, Y., Du, X., Li, C., Wang, M., et al. (2011). Simultaneous determination of five plant growth regulators in fruits by modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction and liquid chromatography–tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 60*(1), 60–65.
Taglienti, A., Sequi, P., Cafiero, C., Cozzolino, S., Ritota, M., Ceredi, G., et al. (2011). Hayward kiwifruits and plant growth regulators: Detection and effects in post-harvest studied by magnetic resonance imaging and scanning electron microscopy. *Food Chemistry, 126*(2), 731–736.
Wasternack, C., & Parthier, B. (1997). Jasmonate-signalled plant gene expression. *Trends in Plant Science, 2*(8), 302–307.
Wasternack, C., Stenzel, I., Hause, B., Hause, G., Kutter, C., Maucher, H., et al. (2006). The wound response in tomato – Role of jasmonic acid. *Journal of Plant Physiology, 163*(3), 297–306.
Wu, Y., & Hu, B. (2009). Simultaneous determination of several phytohormones in natural coconut juice by hollow fiber-based liquid–liquid–liquid microextraction-high performance liquid chromatography. *Journal of Chromatography A, 1216*(45), 7657–7663.
Xie, S., Wang, F., & Chen, Z. (2013). Determination of endogenous jasmonic acid in plant samples by liquid chromatography-electrochemical detection based on derivatization with dopamine. *Analyst, 138*(4), 1226–1231.
Xiong, X. J., Rao, W. B., Guo, X. F., Wang, H., & Zhang, H. S. (2012). Ultrasensitive determination of jasmonic acid in plant tissues using high-performance liquid chromatography with fluorescence detection. *Journal of Agricultural and Food Chemistry, 60*(20), 5107–5111.
Xue, J., Wang, S., You, X., Dong, J., Han, L., & Liu, F. (2011). Multi-residue determination of plant growth regulators in apples and tomatoes by liquid chromatography/tandem mass spectrometry. *Rapid Communications in Mass Spectrometry, 25*(21), 3289–3297.
Yu, Z., Kabashima, T., Tang, C., Shibata, T., Kitazato, K., Kobayashi, N., et al. (2010). Selective and facile assay of human immunodeficiency virus protease activity by a novel fluorogenic reaction. *Analytical Biochemistry, 397*(2), 197–201.
Zhang, Y., Li, Y., Hu, Y., Li, G., & Chen, Y. (2010). Preparation of magnetic indole-3-acetic acid imprinted polymer beads with 4-vinylpyridine and β-cyclodextrin as binary monomer via microwave heating initiated polymerization and their application to trace analysis of auxins in plant tissues. *Journal of Chromatography A, 1217*(47), 7337–7344.