Talanta 99 (2012) 420–425

Contents lists available at SciVerse ScienceDirect

Talanta

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

A new derivatization approach for the rapid and sensitive analysis of brassinosteroids by using ultra high performance liquid chromatography-electrospray ionization triple quadrupole mass spectrometry

Feifeng Huo [a], Xin Wang [a], Yehua Han [a], Yu Bai [a,*], Wei Zhang [a], Hancheng Yuan [b], Huwei Liu [a]

[a] Beijing National Laboratory for Molecular Sciences, Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, Institute of Analytical Chemistry, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, PR China
[b] Waters Corporation, Beijing 100026, PR China

ARTICLE INFO

Article history:
Received 1 April 2012
Received in revised form
27 May 2012
Accepted 31 May 2012
Available online 7 June 2012

Keywords:
Brassinosteroids
BPBA
Derivatization
UHPLC
Triple quadrupole mass spectrometry
Arabidopsis thaliana

ABSTRACT

In this work, a new labeling reagent, 2-bromopyridine-5-boronic acid (BPBA), was introduced to derivatize brassinosteroids (BRs). The BPBA not only provided a very simple and rapid labeling procedure, but also remarkably increased the detection sensitivity of BRs. Based on this new labeling reaction, a rapid and sensitive method for BRs' analysis in *Arabidopsis thaliana* was established by using the ultra high performance liquid chromatography-electrospray ionization triple quadrupole mass spectrometry (UHPLC-ESI-QqQ-MS). The extraction and purification procedure of the plant sample was also simplified and improved in this work. Good linearities were obtained for three BRs with the determination coefficients (R^2) about 0.9999. The limits of detection ($S/N=3$) for three BRs were found to be 2.00–8.00 ng/L while the limits of quantification ($S/N=10$) were 6.00–23.0 ng/L. The RSD % for all three samples are lower than 8.67% ($n=5$). The recoveries of three BRs spiked in *A. thaliana* samples were from 76.9% to 86.1%. Using this method, the endogenous 0.055 ng/g fresh weight (FW) 24-epiBR and 0.070 ng/g (FW) 28-epihomoBR were successfully detected from only 2 g *A. thaliana* plants.

1. Introduction

Brassinosteroids (BRs) were first discovered in 1971 and have been confirmed as the sixth class of plant hormones [1,2]. BRs are involved in numerous biological processes of plants, such as inducing cell elongation and division, increasing DNA and RNA polymerase activity, stimulating ethylene production, and enhancing tolerance of drastic environments [3–5]. Compared with other plant hormones, the concentrations of BRs are extremely low. In general, pollens and immature seeds contain BRs in a range of 1–100 ng/g (FW), while the young roots and leaves contain even less BRs in a range of 0.01–0.1 ng/g (FW). Low concentrations along with co-existent intricate matrix aggravate the difficulties for the isolation and the determination of BRs in plants, which induces the ambiguous understanding of their biological synthesis, degradation, metabolic pathways and bio-functions.

Many techniques have been employed for the detection and identification of BRs in various plant species. Since BRs are non-volatile compounds, gas chromatography-mass spectrometry (GC–MS) in combination with the methaneboronic acid derivatization was used in early research [6–10]. However, the experimental procedures were complicated and time-consuming, and the analytical sensitivity was not satisfactory. High performance liquid chromatography (HPLC) has been frequently used for the plant hormones analysis. Since BRs lack suitable chromophores, organic boronic acids derivatizations through the vicinal groups of BRs usually have to be performed when UV detector was employed [11–16]. Compared with HPLC, Immunoassay offered higher sensitivity for the BRs analysis [17,18], but the preparation and the cross-reactivity of antibodies are the major drawbacks. In order to obtain the lower LOD for BRs, new sample preparation method was developed. Most recently, Li's group synthesized porous magnetic polymer beads to selectively extract 24-epiBR, with which 26.2 μg/kg 24-epiBR was found in real breaking-wall rape pollen samples [19].

LC-MS has become an applicable approach for the analysis of BRs in recent years. Gamoh et al. [20] reported a LOD of 2 ng BRs by using LC-MS with atmospheric pressure chemical ionization

* Corresponding author. Tel.: +86 10 62758198; fax: +86 10 62751708.
E-mail address: yu.bai@pku.edu.cn (Y. Bai).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.05.073

(APCI) source in select ion monitoring (SIM) mode. Based on ESI-MS with SIM mode, Svatoš et al. [21] improved the LOD to sub-femtomolar by means of dansyl-3-aminophenylboronic acid derivatization. For the sensitivity improvement of detection approaches based on LC-MS, one major concern is to enhance the ionization efficiency of the analytes by derivatization. In the previous literatures, several boronic acids have been tried for the derivatization of BRs [20,21], but the reactions were relatively complicated and some of them were not cost-effective. In recent years, UHPLC has been widely used for the analysis of different compounds, which holds advantages over the HPLC due to its increased resolution, higher sensitivity, excellent peak shapes and enhanced reproducibility [22–26].

Here in this study, 2-bromopyridine-5-boronic acid (BPBA) was first introduced as a new labeling reagent to derivatize BRs. Compared with the previous reported boronic acids [11–16,20–21], BPBA is chemically stable and commercially inexpensive, derivatization process is also simple and easy to be performed. On the basis of this new labeling approach and the UHPLC-MS method, a rapid, simple and sensitive BRs detection method was established with which the LOD was significantly reached to attomolar level. Meantime, compared with the conventional procedure, the steps of extraction and purification of BRs from plants were also simplified and improved. With this method, the naturally existing 24-epiBR and 28-epihomoBR were successfully detected from only 2 g *Arabidopsis thaliana* plants. All these improvements facilitated the manipulation of samples and shortened the analysis duration, which made them applicable for the real sample analysis.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile (ACN), methanol, formic acid (FA), trifluoroacetic acid (TFA) and acetic acid from Dikma Technology (Richmond, VA, USA) were of HPLC grade. Water (H_2O) was obtained from Hangzhou Wahaha Group Co., Ltd. (Zhejiang, China) and filtrated through a 0.2 μm Isopore™ membrane (Millipore Corporation, Bedford, MA, USA). 24-epiBR, 28-homoBR and 28-epihomoBR were purchased from Shanghai Weidi Biochemistry Limited Company (Nanchang, China) with a purity of about 90%, and BPBA was from J&K CHEMICA Company (Beijing, China) with a purity of 95%. Anhydrous tetrahydrofuran (THF) and chloroform were obtained after dehydration of analytical grade reagent.

2.2. Instrumentation

2.2.1. Equipment and experimental conditions of QToF MS system

Waters Xevo QToF MS (Waters Corporation, Midford MA, USA) was operated at positive mode. The ESI source conditions were optimized as follows: The voltage of capillary, sampling cone and extraction cone was set as 3400 V, 40 V and 4.0 V, respectively; the nebulization gas was set as 1000 L/h at 550° C; the source temperature was set at 100 °C. To ensure the accurate mass analysis, 400 pg/μL leucine–enkephalin was used as lock mass (m/z 556.2771 for positive ion mode) with a flow rate of 5 μL/min. The m/z scan range was from 150 to 1000 with the scan time of 0.2 s. Data acquisition was carried out by MassLynx v 4.1 software.

2.2.2. Equipment and experimental conditions of UHPLC-QqQ MS system

The UHPLC separation was performed on the Waters Acquity™ UPLC system (Waters Corporation, Midford MA, USA), and the analytical column used was an Acquity UPLC™ BEH C18 (1.7 μm, 2.1 mm × 100 mm). The flow rate of mobile phase was set at 0.35 mL/min with column temperature of 35 °C and injection volume of 5 μL. The separation was performed by gradient elution using organic phase (A) was 0.1% formic acid in acetonitrile and the aqueous mobile phase (B) was 0.1% formic acid in water. The gradient elution program was employed during the separation process (Solution A: 70–85% in 0.5 min, 85–95% in 2.5 min, 95% for 1.5 min, 70% for 3.5 min).

The UHPLC system was coupled to a Waters XEVO TQ MS system (Waters Corporation, Milford, MA, USA) in the positive mode. Data was acquired by the multiple reaction monitoring (MRM) mode. The capillary voltage was 3.05 kV. The source block and desolvation temperatures were set at 150° C and 400° C, respectively. The cone gas flow was 50 L/h and the desolvation gas flow was 650 L/h. Argon was used as the collision gas at a pressure of approximately 3.2×10^{-3} mbar. The protonated molecule was chosen as the precursor ion and the most intensive product ion was selected for the quantification. The selected quantification ion plus another specific product ion were chosen for the confirmation. MRM mass spectrometric parameters of the three analytes were summarized in Table 1. Data acquisition was carried out by MassLynx v 4.1 software.

2.3. Derivatization of BRs by BPBA

24-epiBR, 28-homoBR and 28-epihomoBR (Fig. 1) were used to perform the derivatization experiment. 12 mg of each BR and 20 mg of BPBA were weighed together in a glass tube, followed by the addition of 3 mL anhydrous THF. The derivatization reaction was carried out just by shaking the test tube manually for several seconds. The solution was then dried under N_2 reflux to remove the THF. The dried derivatives were dissolved in methanol, and stored at −20° C for further analysis.

2.4. Plant sample treatment

A. thaliana wild type Columbia (Col-0) plants were grown in soil at 22° C with a 16-h-light/8-h-dark cycle in a growth chamber. 10 g leaves of 2–3 weeks old plants were used for BRs isolation. According to the previous methods [18,27,28], fresh

Table 1
Optimized MRM parameters of three BRs by using UHPLC-QqQ MS (Cone voltage [V]; Collision energy [eV]).

Analyte	Quantification					Confirmation			
	Precursor ion (m/z)	Product ion (m/z)	Cone voltage	Collision energy	Dwell time	Product ion (m/z)	Cone voltage	Collision energy	Dwell time
24-epibrassinolide	646.4	226	54	46	0.12	628	54	26	0.12
28-epihomobrassinolide	660.4	226	46	52	0.12	642	46	28	0.12
28-homobrassinolide	660.4	226	46	52	0.12	642	46	28	0.12

plant leaves were ground to a fine powder under liquid nitrogen and extracted in ice-cold 80% (v/v) methanol for 2 h. After centrifugation, supernatant was extracted by Bond Elut Plexa SPE column (0.5 g, 6 mL, Varian, Palo Alto, CA, USA). The column was first conditioned with 10 mL 70% (v/v) ethanol and then equilibrated by 5 mL H_2O and 5 mL 40 mM CH_3COONH_4 (pH 6.5). After sample loading, 15 mL 40 mM CH_3COONH_4 (pH 6.5) was used to wash the column. At last, the sample was eluted by 3 mL methanol. The extract was then diluted by 40 mM ammonium acetate buffer (pH 6.5) and further purified by Centricon (3 kD, 15 mL, Millipore, MA, USA) to screen large molecules and proteins from plant matrix. Subsequently, the supernatants that passed through the membrane were extracted by Strata™ X reversed-phase column (33 μm, 3 mL, Phenomenex, Torrance, CA, USA) which was conditioned by 5 mL methanol and equilibrated by 5 mL H_2O and 5 mL 40 mM CH_3COONH_4 (pH 6.5) in advance. The BRs sample was eluted by 100% methanol from the column. The eluate was dried by N_2 reflux, and then derivatized with BPBA.

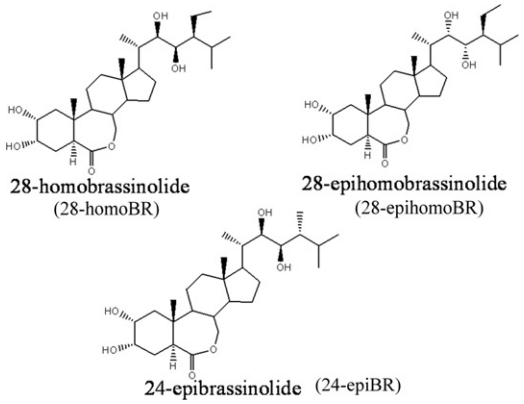

Fig. 1. Chemical structures of three BRs.

2.5. Linearity and recovery test for three BRs

To evaluate the proposed method, the recovery experiments were performed by extraction of 0.5 g ground powder of above plant leaves with the addition of 500 ng/L 24-epiBR, 28-homoBR and 28-epihomoBR. The calibration curves were constructed using standard BRs dissolved in ACN by plotting the peak areas versus the analyte concentrations. 2.0 g plant leaves without the addition of BRs are utilized for the naturally existing BRs detection. The spiked and un-spiked plant samples were then extracted and derivatized following the procedures described above. Recovery of the whole method was calculated according to the linear curve generated from the standards without matrix.

3. Results and discussion

3.1. Optimization of the derivatization procedure

Boronic acids have high affinity to vicinal diols by the formation of boronic esters [29]. As one of the boronic acids, BPBA was firstly reported as the labeling reagent for the detection of diol biomarkers after exposure to the toxic styrene and 1, 3-butadiene [30]. It is important that the nitrogen atom in BPBA can significantly enhance the ionization efficiency during the ESI MS measurement in the positive mode. The characteristic isotopic peaks facilitate the identification of BRs from the complicated matrix. Hereby, we proposed its new application for labeling BRs through the vicinal groups. In the previous literature, the reactions of vicinal hydroxyl groups with organic boronic acids were frequently carried out under following conditions, heating, using solvent of anhydrous THF or chloroform, with or without catalyst of $MgSO_4$ or pyridine [31–38]. In order to find a simple and proper reaction procedure, the solvent and catalysts of the derivatization were systematically optimized in our experiment. The experimental results revealed that, compared with the chloroform and ACN, anhydrous THF offered a better derivatization of three BRs,

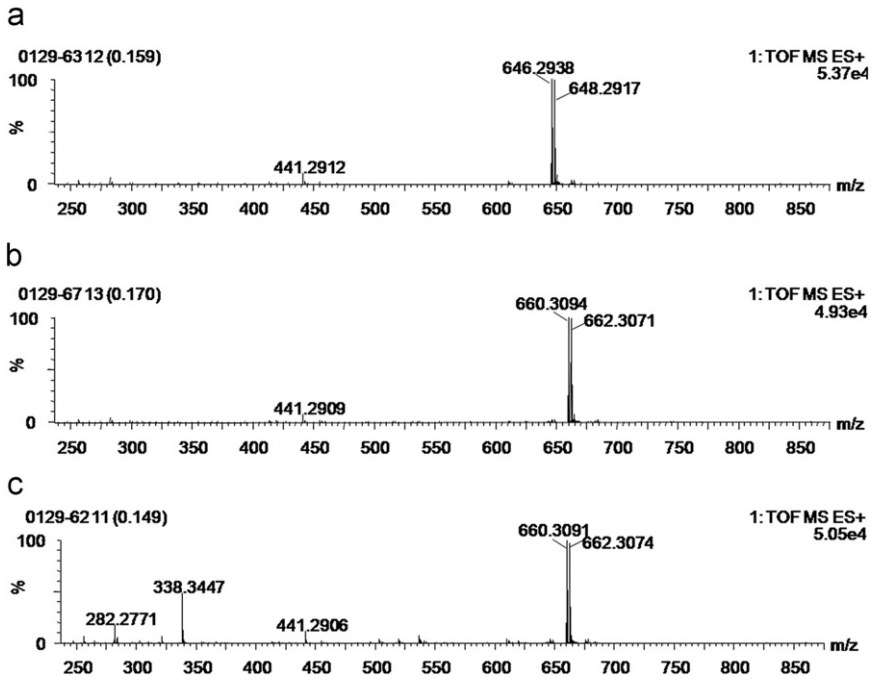

Fig. 2. Full scan mass spectra of the three BPBA derivatized BRs using QToF MS. (a) 24-epiBR; (b) 28-epihomoBR; (c) 28-homoBR.

and no catalyst was required for the derivatization reaction at all. It was found that the reaction took longer time and heating was required when ACN or chloroform was utilized as the solvent. Therefore anhydrous THF solvent without heating and catalyst were confirmed as the derivatization conditions. The obtained derivatives was dried by N_2 reflux, diluted by methanol, and then analyzed by Waters Xevo QToF MS. Fig. 2 is the full scan mass spectra of BPBA derivatized 24-epiBR, 28-epihomoBR and 28-homoBR. The peaks at *m*/*z* 646/648 for 24-epihomoBR and *m*/*z* 660/662 for 28-epihomoBR and 28-homoBR were clearly observed. Moreover, no peaks related to underivatized 24-epibrassinolide, 28-epihomobrassinolide and 28-homobrassinolide were observed (*m*/*z* 480 for 24-epihomobrassinolide and *m*/*z* 494 for 28-epihomobrassinolide and 28-homobrassinolide), which means the reaction is full proceeded. Meantime, no peaks for bi-BPBA derivatized products were detected (No peaks at *m*/*z* 810 for 24-epihomobrassinolide and *m*/*z* 824 for 28-epihomobrassinolide and 28-homobrassinolide), indicating that only the mono-BPBA derivatized products were produced. With the addition of 0.1% formic acid in the mobile phase, no sodiated and potassiated adducts of the three BRs were observed. It was presumed that the reaction was formed on the C22–C23 diol moiety since the reaction at C2–C3 diol moiety was sterically hindered [21] (Fig. 3). The reaction efficiency and the stability of the products were further investigated. 10 μL products was taken from the reaction tube right after the shaking step and kept in −20° C. In the next 2 h, sampling of 10 μL every certain time was carried out. All collected samples were analyzed by using UHPLC-QToF MS. Results showed that no difference in the peak area of the mono-BPBA derivative and no other derivatives were observed, which indicated that the reactions have been completed in several seconds effectively. Additional experiments showed that derivatives kept stable for more than two months at −20° C. Compared with the previously reported methods that required a temperature of 60–75° C, duration of 20–30 min and catalyst of pyridine, our BPBA method can be completed in several seconds at room temperature with no need of any catalyst. The BPBA method shows obvious superiority for rapid and convenient analysis of BRs.

3.2. Optimization of chromatographic separation

The mixture of three BR derivatives (5 μg/L each) was used in the optimization of chromatographic separation. Different mobile phases (methanol and ACN) and additives (FA, acetic acid and TFA at various concentrations) were tested. Considering the resolution and analysis speed, a Waters BEH column (100 mm × 2.1 mm, 1.7 μm) was chosen to perform the separation of three BRs. Compared with mobile phase of methanol/water, ACN/water offered a better separation of those three compounds. The ratio of ACN and water was subsequently optimized. For the investigation of additives in mobile phase, FA provided more sensitive results than acetic acid under the ESI positive mode. The best signal response was obtained when 0.1% FA was added. When the water proportion of the mobile phase was increased, a better chromatographic resolution was achieved. But high water content brought the response decrease of ESI MS. Considering on this point, gradient elution was chosen at last.

3.3. UHPLC-QqQ MS method for the analysis of BRs

28-homoBR and 28-epihomoBR are diastereomers, and 24-epiBR is their homolog with only one-CH_2 difference, so it is very difficult

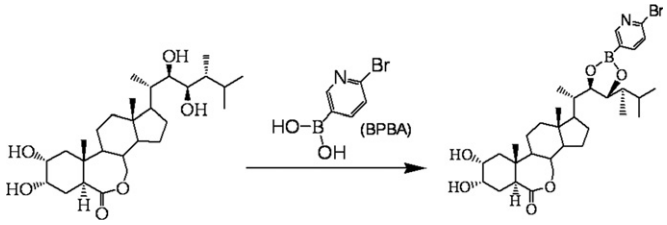

Fig. 3. Proposed derivatiztion reaction of 24-epiBR by BPBA.

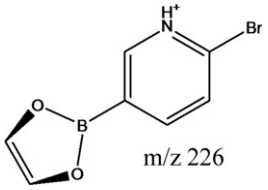

Fig. 5. Proposed structure of the quantification ion at *m*/*z* 226 for 24-epihomoBR, 28-epihomoBR and 28-homoBR.

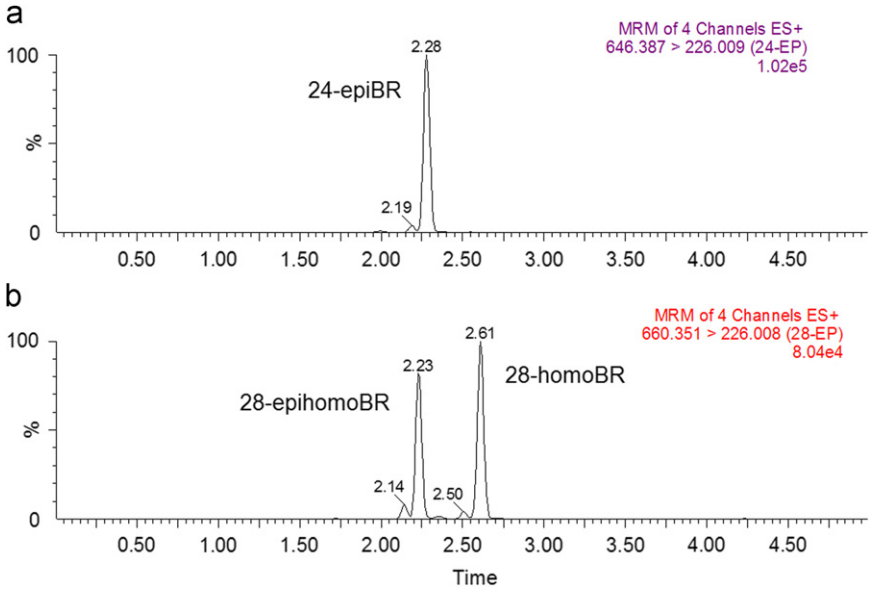

Fig. 4. MRM chromatograms of the spiked sample of three derivatized BRs using UPLC-QqQ MS. (a) 24-epiBR; (b) 28-epihomoBR and 28-homoBR.

to separate these three BRs in a single run using only chromatographic approach. Considering both resolution and sensitivity, the combination of UHPLC with MS may potentially differentiate the three compounds. Fig. 4 shows the MRM chromatogram of the three derivatized BRs. The peak at 2.28 min (Fig. 4a), 2.23 min and 2.61 min (Fig. 4b) corresponds to 24-epiBR, 28-epihomoBR and 28-homoBR, respectively. Peaks at 2.19 min (Fig. 4a), 2.14 min and 2.50 min (Fig. 4b) were from the isomeric impurities. The existing impurities limited the flow rate of mobile phase and restricted the performance of UHPLC system because the increase of the flow rate induced the decrease of the peak resolution. Nevertheless, optimized flow rate at 0.35 mL/min provided the satisfactory analysis results.

The mass spectrometric parameters including capillary voltage, cone voltage, source temperature, desolvation temperature, cone gas flow and desolvation gas flow were all optimized (see details in Section 2.2.2). The MRM parameters of the established UHPLC-QqQ-MS method are shown in Table 1. The qualitative ions of 24-epiBR is at m/z 628 while the qualitative ions of 28-epihomoBR and 28-homoBR are at m/z 642, which were generated by one H_2O loss from the $[M+H]^+$. The proposed structures of the quantification ion at m/z 226 for 24-epiBR, 28-epihomoBR and 28-homoBR are shown in Fig. 5.

A series of standard mixtures of 24-epiBR, 28-epihomoBR and 28-homoBR were prepared for the method validation. Mean areas ($n=5$) generated from the standard solutions were plotted against the BRs concentration to establish calibration equations. Good linearities were obtained for three BRs with the coefficients (R^2) about 0.9999 and the relative standard deviation (RSD) lower than 8.67% ($n=5$) (Table 2). Limits of detection (LODs) and limits of quantification (LOQs) of the proposed method were determined as the analytes concentration corresponding to signal-to-noise ratio (S/N) of 3 and 10 from standard BRs dissolved in ACN. In this respect, the LODs of three BRs are 2.00 ng/L for 24-epibrassinolide, 6.00 ng/L for 28-epihomobrassinolide, and 8.00 ng/L for 28-homobrassinolide. In addition, the LOQs of three BRs are 6.00 ng/L for 24-epibrassinolide, 19.0 ng/L for 28-epihomobrassinolide, and 23.0 ng/L for 28-homobrassinolide. As the injection volume is only 5 μL, the absolute LODs of the three BRs can be greatly improved to attomolar level, which are so far the most sensitive method and quite promising for the analysis of BRs in real plant samples.

3.4. Recovery test and method application

Because of the extremely low concentration of BRs and co-existent intricate matrix, the workload for the extraction of BRs from plant is rather laborious and complicated in previous literatures. Generally, the crude ground plant materials were first extracted by methanol and the extract were then partitioned by chloroform and water. The chloroform extract was further separated by silica gel chromatography with gradient elution with chloroform and methanol. The target partition was separated by using the DEAE ion-exchange chromatography. Finally, prior to the derivatization procedure, the extracts were subjected to a preparative HPLC [9,14,39–46]. It is noteworthy that rice lamina inclination test [47–54] was always required to guide the extraction during the silica gel chromatographic separation. All of these make the whole procedure quite time-consuming. Swaczynova [18] later simplified the procedure by applying solid phase extraction, but the DEAE ion-exchange chromatography still made the extraction inconvenient and expensive. Here, we further improve the plant treatment process by first introducing Centricon filter tube to replace DEAE ion-exchange chromatography, which dramatically reduced the duration of BRs extraction from days to less than 6 h. Based on the improved methods, the mean recovery values were calculated as 85.7%, 76.9% and 86.1% for 24-epiBR, 28-epihomoBR, and 28-homoBR, respectively when 0.5 g ground powder of plant leaves was utilized (Table 2).

It should be noted that, in the previous literature, several kilograms fresh plant materials were required to ensure the detection of BRs [6–17,20], which aggravated the difficulties for their extraction and purification. Using this proposed method, the naturally existing 0.055 ng/g (FW) 24-epiBR ($S/N=29.3$) and 0.070 ng/g (FW) 28-epihomoBR ($S/N=10.4$) were successfully detected from only 2 g *A. thaliana* plants (see Fig. 6). The 28-homobrassinlode was not detected in the plant sample, which

Table 2
Linear regression equation and LOD data of BRs analyzed by UHPLC-QqQ MS.

Analyte	Retention time (min.)	Linear range (ng/L)	Equation of linear regression	r^2	RSD ($n=5$) (%)	LOD (ng/L)	LOQ (ng/L)	Recovery ($n=3$) (%)
4-epiBR	2.28	10–10,000	$y=948.3x-0.14$	0.9999	4.43	2.00	6.00	85.7
28-epihomoBR	2.23	20–10,000	$y=597.2x-0.31$	0.9999	8.24	6.00	19.0	76.9
28-homoBR	2.61	50–10,000	$y=759.6x+9.77$	0.9999	8.67	8.00	23.0	86.1

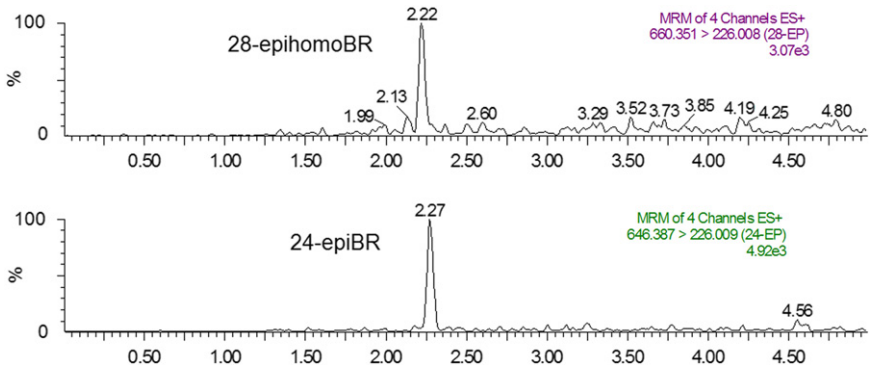

Fig. 6. MRM chromatograms of BPBA derivatized BRs from 2.0 g *Arabidopsis thaliana* plant sample using UHPLC-QqQ-MS.

might cause by the sensitivity of the method and different species of plant samples.

4. Conclusions

A rapid and simple derivatization approach for the sensitive analysis of BRs has been proposed in this study. The qualification of BRs can be facilitated by the unique bromine isotopic peaks coming from BPBA during the mass spectrometric analysis. The sensitivity can be enhanced to the attomolar level by the BPBA derivatization combined with the usage of UHPLC-QqQ MS. Compared with previous sample pretreatment, the simplified procedure proposed in this paper provides a high efficient strategy for the BRs analysis from complicate plant materials. The naturally existing 24-epiBR and 28-epihomoBR were successfully detected from only 2 g *A. thaliana* plants. This method offers easier manipulation of samples, less analysis duration and opportunities for the endogenous BRs analysis from less amount of real sample.

Acknowledgments

This study was financially supported by the National Natural Science Foundation of China (Grant nos. 20805001, 91132717 and 20115008) and the Project Sponsored by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry. The authors also appreciate Ms. Hui Qi and Mr. Xing Wen for the kind donation of *A. thaliana*, Mr. Zhenhua Zhao, Mr. Hongliang Wu, Ms Lanying Zhang and Mr. Tianwen Sun for supplying the anhydrous THF and purified chloroform.

References

[1] J.W. Mitchell, N. Mandava, J.F. Worley, J.R Plimmer, M.V. Smith, Nature 225 (1970) 1065–1066.
[2] M.D. Grove, G.F. Spencer, W.K. Rohwedder, N. Mandava, J.F Worley, J.D. Warthen Jr., G.L. Steffens, J.L. Flippen-Anderson, J.C. Cook Jr., Nature 281 (1979) 216–217.
[3] A. Bajguza, A. Tretynb, Phytochemistry 62 (2003) 1027–1046.
[4] G. Adam, A. Porzel, J. Schmidt, B. Schneider, B. Voigt, Stud. Nat. Prod. Chem. 18 (1996) 495–549.
[5] G. Adam, J. Schmidt, B. Schneider, Prog. Chem. Org. Nat. Prod. 78 (1999) 1–46.
[6] S. Takatsuto, J. Chromatogr. A 658 (1994) 3–15.
[7] S. Takatsuto, B. Ying, M. Morisaki, N. Ikekawa, J. Chromatogr. A 239 (1982) 233–241.
[8] N. Ikekawa, S. Takatsuto, T. Kitsuwa, H. Saito, T. Morishita, H. Abe, J. Chromatogr. A 290 (1984) 289–302.
[9] J. Schmidt, B. Spengler, T. Yokota, M. Nakayama, S. Takatsuto, B. Voigt, G. Adam., Phytochemistry 38 (1995) 1095–1097.
[10] J. Schmidt, T. Altmann, G. Adam, Phytochemistry 45 (1997) 1325–1327.
[11] K. Gamoh, T. Kitsuwa, S. Takatsuto, Anal. Sci. 4 (1988) 533–535.
[12] K. Gamoh, S. Takatsuto, Anal. Chim. Acta. 222 (1989) 201–204.
[13] K. Gamoh, K. Omote, N. Okamoto, J. Chromatogr. A 469 (1989) 424–428.
[14] K. Gamoh, H. Sawamotoa, S. Kakatsutob, Y. Watabec, H. Arimoto, Anal. Chim. Acta. 228 (1990) 101–105.
[15] J. Wintera, B. Schneiderb, S. Meyenburga, D. Stracka, G. Adama, Phytochemistry 51 (1999) 237–242.
[16] K. Gamoh, H. Sawamoto, S. Ktakatsuto, Y. Watabec, H. Arimotoc, J. Chromatogr. A 515 (1990) 227–231.
[17] T. Yokota, S. Watanabe, Y. Ogino, J. Plant Growth Regul. 9 (1990) 151–159.
[18] J. Swaczynova, O. Novak, E. Hauserova, K. Fuksova, M. Sisa, L. Kohout, M. Strnad, J. Plant Growth Regul. 26 (2007) 1–14.
[19] Z.M. Zhang, Y. Zhang, W. Tan, G.K. Li, Y.L. Hu, J. Chromatogr. A 1217 (2010) 6455–6461.
[20] K. Gamoh, H. Abe, K. Shimada, S. Takatsuto, Rapid Commun. Mass Spectrom. 10 (1996) 903–906.
[21] A. Svatoš, A. Antonchick, B. Schneider, Rapid Commun. Mass Spectrom. 18 (2004) 816–821.
[22] A. Nordstrom, G. OMaille, C. Qin, G. Siuzdak, Anal. Chem. 78 (2006) 3289–3295.
[23] Y.F. Shen, R. Zhang, R.J. Moor, J. Kim, T.O. Metz, K.K. Hixson, R. Zhao, E.A. Livesay, H.R. Udseth, R.D. Smith, Anal. Chem. 77 (2005) 3090–3100.
[24] I.D. Wilson, J.K. Nicholson, J. Castro-Perez, J.H. Granger, K.A. Johnson, B.W. Smith, R.S. Plumb, J. Proteome Res. 4 (2005) 591–598.
[25] M.I. Churchwell, N.C. Twaddle, L.R. Meeker, D.R. Doerge, J. Chromatogr. B 825 (2005) 134–143.
[26] J. Castro-Perez, R. Plumb, J.H. Granger, I. Beattie, K. Joncour, A. Wright, Rapid Commun. Mass Spectrom. 19 (2005) 843–848.
[27] T. Yokota, T. Nomura, M. Nakayama., Plant Cell Physiol. 38 (1997) 1291–1294.
[28] T. Nomura, M. Nakayama, J.B. Reid, Plant Physiol. 113 (1997) 31–37.
[29] J. Yan, H. Fang, B. Wang, Med. Res. Rev. 25 (2005) 490–520.
[30] S.J. Shen, F. Zhang, S. Zeng, J. Zheng, Anal. Biochem. 386 (2009) 186–193.
[31] C.D. Roy CD, H.C. Brown, Monatsh. Chem. 138 (2007) 879–887.
[32] J.M. Thomson, A.M. Distler, F. Prati, R.A. Bonomo, J. Biol. Chem. 281 (2006) 26734–26744.
[33] S. Morandi, E. Caselli, A. Forni, M. Bucciarelli, G. Torre, F. Prati, Tetrahedron: Asymmetry 16 (2005) 2918–2926.
[34] S. Superchi, D. Casarini, C. Summa, C. Rosini, J. Org. Chem. 69 (2004) 1685–1694.
[35] J.W.J. Kennedy, D.G. Hall, J. Organomet. Chem. 680 (2003) 263–270.
[36] F. Morandi, E. Caselli, S. Morandi, P.J. Focia, J. Blazquez, B.K. Shoichet, F. Prati, J. Am. Chem. Soc. 125 (2003) 685–695.
[37] D.G. Hall, Boronic Acids: Preparation and Applications in Organic Synthesis and Medicine, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2005 80–93.
[38] S.M. Resnick, D.S. Torok, D.T. Gibson, J. Org. Chem. 60 (1995) 3546–3549.
[39] T. Hai, B. Schneider, G. Adam, Phytochemistry 40 (1995) 443–448.
[40] T. Hai, B. Schneider, A. Porzel, G. Adam, Phytochemistry 41 (1996) 197–201.
[41] T. Nomura, M. Nakayama, J.B. Reid, Y. Takeuchi, T. Yokota, Plant Physiol. 113 (1997) 31–37.
[42] T. Yokota, T. Nomura, M. Nakayama, Plant Cell Physiol. 38 (1997) 1291–1294.
[43] D. Gupta, R. Bhardwaj, P.K. Nagar, S. Kaur, Plant Growth Regul. 43 (2004) 97–100.
[44] R. Bhardwaj, S. Kaur, P.K. Nagar, H.K. Arora, Plant Growth Regul. 53 (2007) 1–5.
[45] T. Yokota, J. Baba, S. Koba, N. Takahashi, Agric, Biol. Chem. 48 (1984) 2529–2534.
[46] L. Dinana, J. Harmathab, R. Lafont, J. Chromatogr. A 935 (2001) 105–123.
[47] M. Arima, T. Yokota, N. Takahashi, Phytochemistry 23 (1984) 1519–1587.
[48] K. Wada, S. Marumo, H. Abe, T. Morishita, K. Nakamura, M. Uchiyama, K. Mori, K Agric., Biol. Chem. 48 (1984) 719–726.
[49] B. Voigt, A. Porzel, C. Bruhn, C. Wagner, K. Merzweiler, G. Adam, Tetrahedron 53 (1997) 17039–17054.
[50] K. Wada, S. Marumo, N. Ikekawa, N. Morisaki, K. Mori, Plant Cell Physiol. 22 (1981) 323–325.
[51] T. Yokota, T. Matsuoka, T. Koarai, M. Nakayama, Phytochemistry 42 (1996) 509–511.
[52] A. Friebe, A. Volz, J. Schmidt, B. Voigt, G. Adam, H. Schnabl, Phytochemistry 52 (1999) 1607–1610.
[53] T Watanabe, T. Noguchi, T. Yokota, K. Shibata, H. Koshino, H. Seto, S. Kim, S. Takatsuto, Phytochemistry 58 (2001) 343–349.
[54] B. Voigt, P. Whiting, L. Dinan, Cell. Mol. Life Sci. 58 (2001) 1133–1140.