Analyst

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Cite this: *Analyst*, 2013, **138**, 1226

Determination of endogenous jasmonic acid in plant samples by liquid chromatography-electrochemical detection based on derivatization with dopamine

Shanshan Xie, Fang Wang and Zilin Chen*

Jasmonic acid (JA), a type of plant hormone, is widely distributed in a variety of higher plants at very low concentrations, usually several nanograms per gram (ng g^{-1}) fresh weight of the plant tissue. The determination of endogenous JA is challenging work. The typical electrochemical oxidation behavior of JA could only be achieved under extreme conditions such as strongly acidic medium (H_2SO_4) and high applied working potential (1.4–1.6 V), which cannot be used in the electrochemical detection for liquid chromatography (LC). To realize electrochemical detection for LC separation, a mild supporting electrolyte for JA oxidation is required, as the supporting electrolyte solution also serves as the mobile phase of LC. Thus, a novel electrochemical derivatization with dopamine (DA) has been developed and successfully applied to the analysis of endogenous JA in wintersweet flowers and rice florets by liquid chromatography coupled with electrochemical detection (HPLC-ECD). Under optimized experimental conditions including a detection potential of +0.90 V, and 0.04 mol L^{-1} acetate buffer solution (pH 5.07) : acetonitrile (67 : 33, v/v) as the mobile phase, the contents of JA in wintersweet flowers and rice florets were respectively determined to be 7.86 μg g^{-1} and 308 ng g^{-1}, consulting the linear relationship between the peak area of JA–DA derivatives and the standard JA concentration (1.0×10^{-7} mol L^{-1} to 2.0×10^{-5} mol L^{-1}, $R = 0.9986$) with a detection limit of 5.0×10^{-8} mol L^{-1} ($S/N = 3$). The results were consistent with those by LC-UV and LC-MS methods in our group, indicating that this novel pre-column electrochemical derivatization method is feasible.

Received 9th October 2012
Accepted 11th December 2012

DOI: 10.1039/c2an36455g

www.rsc.org/analyst

Introduction

Jasmonates (JAs) are a new family of plant hormones that are widely distributed in a variety of higher plants. As one of the most representative JAs, free jasmonic acid (JA) was first isolated from the culture filtrate of fungi (*Lasiodiplodia theobromae*) in 1971.[1] Since then, plant physiologists have been devoted to researching the physiological activities of JA. It was reported that exogenous JA could affect the germination of seeds (*Dioscorea alata*),[2] including an inhibition effect at high concentrations and promotion effect at low concentrations. JA can also accelerate the division and enlargement of plant cells.[3] Satoru and Kayoka[4] found that JA can promote the maturation of grape berries through stimulating cell division. In addition, JAs were also found to have activities[5–7] against types of cancer including breast cancer, prostate cancer, melanoma, lymphoblastic leukemia and lymphoma cells.[8] However, endogenous JA occurs in the form of enantiomers at very low concentrations, around nanograms per gram fresh weight of the plant tissue. As a result, the qualitative and quantitative analysis of endogenous JA becomes a very challenging task.

In recent years, many methods have been used for the analysis of JAs. Chromatographic and spectroscopic methods have been the most widely used.[9–17] Besides, some bioanalytical means such as radio immunoassay (RIA)[18] and enzyme-linked immunosorbent assay (ELISA)[19] were also reported. However, these methods either used expensive instruments or had insufficient sensitivity for JA analysis in higher plants. In order to develop new methods with low cost and high sensitivity, researchers turned attention to electrochemical methods. The most significant features of electrochemical methods are their convenience, low cost and high sensitivity. Electrochemical detection (ECD) coupled with high performance liquid chromatography (HPLC) could improve the selectivity of electrochemical analysis. Therefore, the HPLC-ECD method has attracted the interest of scientists in the trace analysis of biological samples, especially plant extracts.

In recent years we have worked on the development of new analytical methods for the analysis of endogenous jasmonates. We have successfully developed solid phase extraction materials for the highly selective concentration of JA and clean-up of interference for the pre-treatment of real samples,[21] and a novel method for enhancement of mass spectrometric detection

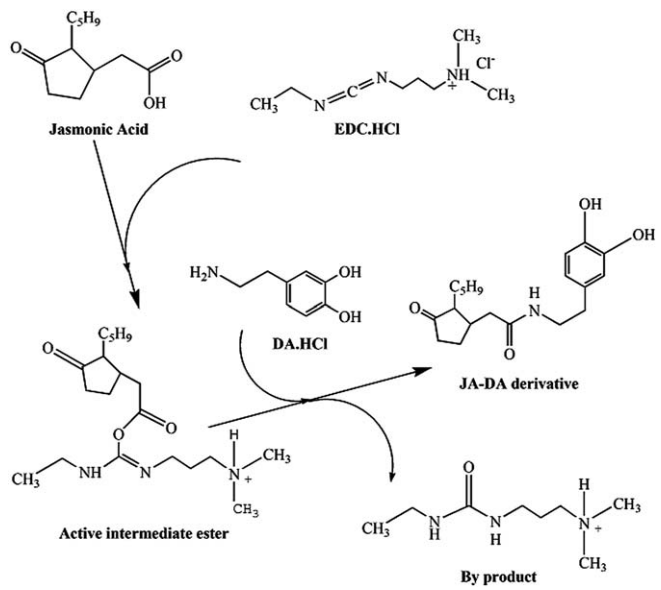
School of Pharmaceutical Sciences, Wuhan University, Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (Wuhan University), Ministry of Education, Wuhan, China. E-mail: chenzl@whu.edu.cn; Fax: +86-27-68759850; Tel: +86-27-68759893

sensitivity.[22] Our previous work[20] reported that electrochemical analysis of methyl jasmonate (MeJA) can be achieved under extreme conditions such as strongly acid medium (H_2SO_4) and applied high working potential (1.4–1.6 V) because of the low electrochemical activity of JA. The low electrochemical activity results in low detection sensitivity. Oxidizing conditions in strongly acidic media means that the supporting electrolyte solution cannot be used as the mobile phase of LC separation, because the strongly acidic condition will destroy the chromatographic column when developing a LC-ECD method. Thus, in order to develop a LC-ECD method for analysis of endogenous JA, we have to solve two major bottleneck problems. One is the enhancement of JA electroactivity so as to have sufficient sensitivity for electrochemical detection. Another is to realize the oxidation of JA under mild conditions so that the supporting electrolyte solution for electrochemical detection can be used as the mobile phase for LC separation. In the present work, we report our strategy for solving these problems by pre-column derivatization of JA with the electrochemically active dopamine. After derivatization of JA with dopamine, both the detection sensitivity and the oxidation potential of JA are improved as expected. Based on the derivatization, a novel method for the analysis of JA has been developed by HPLC-ECD on a bare glassy carbon electrode (GCE). Finally, the method has been successfully applied to analysis of the endogenous JA in plant extracts including wintersweet flowers and rice florets.

Results and discussion

Derivatization of JA

A reaction mechanism for the derivatization of JA with DA is provided in Scheme 1. The commercial compound JA was used as the starting material. Firstly, the carboxyl group of JA was activated by EDC·HCl to obtain the active intermediate ester. Then, this active intermediate ester was transformed into a JA–DA derivative by reacting with DA·HCl. The JA–DA derivative was further characterized in the following experiments by ECD and LC-MS. The reaction efficiency was 69.7%, obtained by calculating the differences in the peak areas of jasmonic acid before and after reaction.



Scheme 1 The reaction mechanism using EDC as the condensing agent.

Electrochemical behavior of the JA–DA derivative

The electrochemical behaviors of the amide derivative of JA (JA–DA) were investigated by differential pulse voltammetry (DPV) in 0.1 mol L^{-1} phosphate buffer (PB, pH = 7.0) on a bare glassy carbon electrode (GCE). The results are shown in Fig. 1. In the presence of EDC·HCl, only one obvious oxidation peak at about +0.90 V can be observed at the bare GCE (curve 1). When DA·HCl was added into the supporting electrolyte containing EDC·HCl, a new peak appears at about +0.12 V (curve 2). However, two new oxidation peaks are respectively observed at about 0.44 V and 0.65 V (*vs.* Ag/AgCl, curve 3) when the JA–DA derivative was introduced into the electrolyte and measured. It is obvious that the new oxidation peaks are due to the electrochemical oxidation of the JA–DA derivative, which can be used as a target for electrochemical detection. Meanwhile, these results suggest the success of the derivatization reaction between JA and DA·HCl in the presence of EDC·HCl.

The sensitivity of this method depends on derivatization efficiency, which is determined by many factors such as the amount of reagents, reaction time and temperature. To obtain the best efficiency, the derivatization conditions were examined. Firstly, the influence of the amount of DA·HCl on the derivatization was investigated. In the presence of 10 μl of standard JA stock solution, the peak area of the JA–DA derivative increased slowly with the increase of DA·HCl concentration from 2.0×10^{-3} to 4.0×10^{-3} mol L^{-1}, reached a maximum at 4.0×10^{-3} mol L^{-1}, and then dropped. As a result, 4.0×10^{-3} mol L^{-1} DA·HCl was selected as the experimental concentration. Secondly, EDC·HCl was used for the condensation of carboxylic acids with amines. The effect of EDC·HCl concentration on the peak area was also examined. When the

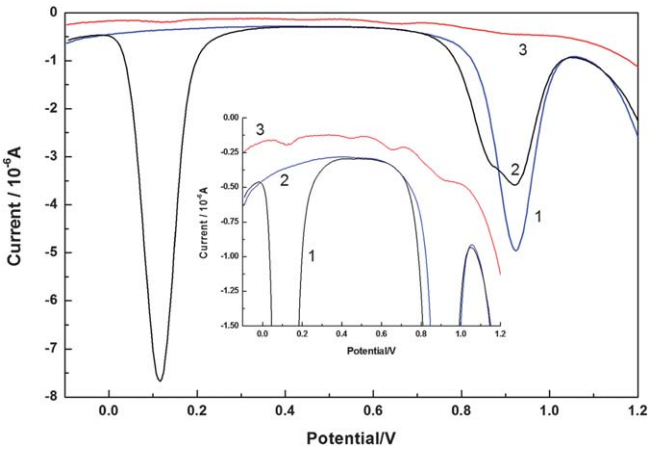


Fig. 1 Differential pulse voltammograms of EDC·HCl (curve 1), EDC·HCl and DA·HCl (curve 2) and JA–DA derivative (curve 3) on a bare GCE in PB solution (pH = 7.0) at a scan rate of 100 mV s^{-1}.

concentration of EDC·HCl was changed from 2.6×10^{-4} to 7.8×10^{-2} mol L^{-1}, the peak area first increased and then decreased. The maximum value was obtained at an EDC·HCl concentration of 2.6×10^{-3} mol L^{-1}. Thirdly, the relationship between reaction time and peak area was studied. Generally, the longer the reaction time, the higher the efficiency of the derivatization reaction. Considering operation convenience and the slow increase of peak area after 12 h, 12 h was selected as the final reaction time. Lastly, the dependence of derivatization efficiency on reaction temperature was evaluated in the range of 40 °C to 70 °C. The peak area of the derivative noticeably increased with the rise in reaction temperature, reached a maximum at 60 °C, and then dropped. Therefore, the derivatization of JA with DA·HCl was carried out at 60 °C.

HPLC-ECD analysis of JA–DA

A typical chromatogram for the separation of the JA–DA derivative and other reactants with electrochemical detection is shown in Fig. 2. In the control experiment (curve 2), only one peak with a retention time of about 410 s can be observed, which was confirmed not to be the JA–DA derivative, and may be due to the electrochemical oxidation of the DA derivative as a by-product. A comparison was made between curve 2 (without JA) and curve 1 (with JA). It was found that a well-defined peak appears at about 1210 s, which was confirmed by LC-MS to be the JA–DA derivative. This peak at about 1210 s was used for further qualitative and quantitative analysis of endogenous JA.

In order to obtain high sensitivity, the chromatographic conditions were optimized. First of all, the proportion of acetonitrile in the mobile phase was investigated. The results showed that the retention time of the JA–DA derivative gradually reduced with the change of acetonitrile percentage from 25% to 60%. An excessively high proportion of acetonitrile was not beneficial to complete separation of the JA–DA derivative in real samples, because the crude extracts of plant tissues were very complicated without too much purification pre-treatment. At the same time, the retention time was too long when the percentage of acetonitrile was less than 30%. Considering the separation efficiency and accuracy, 33% acetonitrile was used for analysis.

Some electrolyte solutions must be introduced into the mobile phase for electrochemical detection. Therefore, different buffer solutions with different pH values were examined including Na_2HPO_4–KH_2PO_4, $C_6H_8O_7$–NaOH and NaAc–HAc. The results showed that the former two electrolytes (Na_2HPO_4–KH_2PO_4 and $C_6H_8O_7$–NaOH) resulted in high background noise so that the peaks of the analytes could not be well-defined. Therefore, NaAc–HAc buffer solution was preferred to serve as the electrolyte. Subsequently, the concentration and pH of NaAc–HAc buffer were checked in the ranges from 0.01 mol L^{-1} to 0.05 mol L^{-1} and from pH 4.0 to pH 6.0, respectively. According to experimental data, 0.04 mol L^{-1} of NaAc–HAc buffer (pH 5.1) was chosen as the optimal electrolyte. It is worth mentioning that little change could be observed when the pH changed from 4.8 to 5.6, but obvious differences appeared outside of that range. For example, the retention time became longer when the pH was less than 4.8, but shorter when it was greater than 5.6, and the peak shape was not good as well. Sometimes, overlap of the target peak would occur, especially in the analysis of plant samples. Based on the consideration of the retention times, resolution and sensitivity, the mobile phase consisted of 0.04 mol L^{-1} acetate buffer solution adjusted to pH 5.1 and acetonitrile (67 : 33, v/v).

Likewise, the flow rate of the mobile phase was also evaluated from 0.60 mL min^{-1} to 1.20 mL min^{-1}. The peak width decreased when the flow rate increased from 0.60 mL min^{-1} to 0.80 mL min^{-1}. However, overlap of peaks occurred with further increase of the flow rate, which would be unfavorable in the separation of real samples. 0.80 mL min^{-1} was used as the optimum flow rate, because of both short analysis times and acceptable resolution for separation of JA–DA and other substances.

As far as the working potential was concerned, this could significantly influence the peak areas of the JA–DA derivative. As

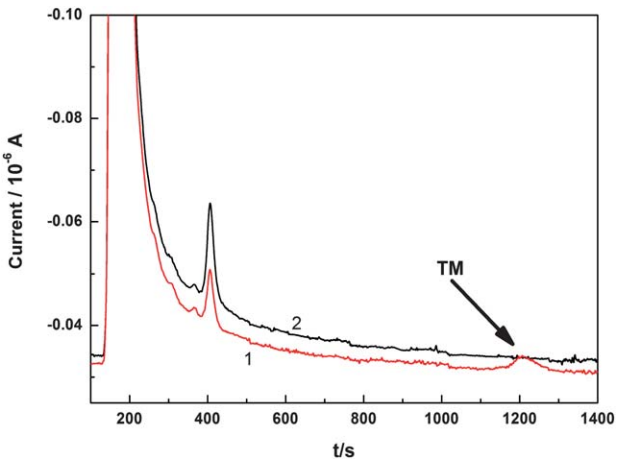


Fig. 2 Chromatograms of derivatization of JA (1.0×10^{-5} mol L^{-1}) with DA (curve 1), and blank control without JA (curve 2). Mobile phase: 0.04 mol L^{-1} acetate buffer solution (pH 5.1) and acetonitrile (67 : 33, v/v). Flow rate: 0.80 mL min^{-1}. Applied potential: +0.90 V.

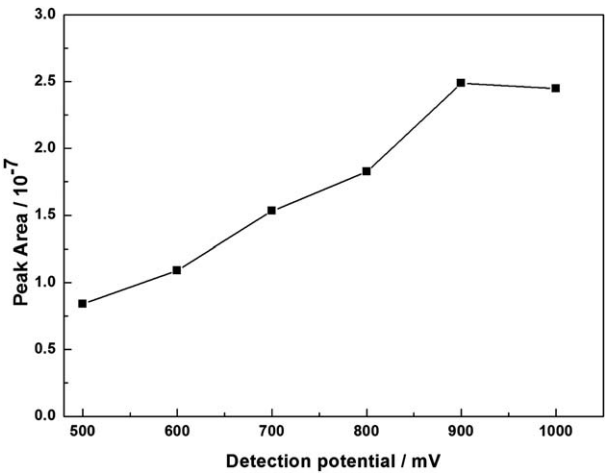


Fig. 3 Effect of detection potentials on the peak area of JA–DA derivative. Mobile phase: 0.04 mol L^{-1} acetate buffer solution (pH 5.1) and acetonitrile (67 : 33, v/v). Flow rate: 0.80 mL min^{-1}.

shown in Fig. 3, the target peak area gradually increased in the range of 0.50 V to 0.90 V, and achieved the maximum value at 0.90 V. Further increase of applied potential would bring about increased baseline noise instead of significant change in peak area. Therefore, amperometric *i–t* curves of the samples for chromatograms were recorded at a working potential of 0.90 V.

Method validation

Different concentrations of standard JA solution were used for derivatization under optimized conditions, and the HPLC-ECD analysis results indicated that the peak area of the JA–DA derivative was linearly dependent on the JA concentration. A calibration curve was characterized under experimental conditions with a mobile phase of 0.04 mol L^{-1} acetate buffer solution (pH 5.1) and acetonitrile (67 : 33, v/v) at flow rate of 0.80 mL min^{-1} and applied potential of +0.90 V. The relationship between peak area of JA–DA derivative and JA concentration could be described by the following linear regression equation in the concentration range of 1.0×10^{-7} to 2.0×10^{-5} mol L^{-1}: Q (nA s) = 0.605 + 0.202c (μmol L^{-1}) ($R = 0.9986$, $n = 9$), where the c is the concentration of derivative, and Q stands for the chromatographic peak area.

A detection limit of 5.0×10^{-8} mol L^{-1} JA was obtained by determining $S/N = 3$. It is obvious that the sensitivity has been greatly improved compared with our previous work.[20]

The inter-day and intra-day precision was investigated to evaluate the precision and accuracy of this method. The data is demonstrated in Table 1. Three different JA concentrations of 1.0×10^{-5} mol L^{-1}, 1.0×10^{-6} mol L^{-1}, and 1.0×10^{-7} mol L^{-1} were used for derivatization under optimized conditions. The relative standard deviation (R.S.D.) for 6 times parallel analysis in one day are 1.48%, 2.56% and 4.94% for the intra-day precision, and 1.76%, 2.87% and 5.48% for the inter-day precision over three days, respectively, suggesting excellent reproducibility of this new method.

Sample analysis

For the sake of reliability of this new method, it was applied to the analysis of endogenous JA in wintersweet flowers and rice florets, and then the recovery was tested as well. The extracts from wintersweet flowers were treated by derivatization and then characterized by LC-MS (Fig. 4). The comparison was made between standard JA (curve a) and endogenous JA in wintersweet flowers (curve b). The two target peaks have similar retention times. The base peaks in the mass spectra are the addition of hydrogen ion $[M + H]^+$ at m/z 346 for JA–DA. All these

Table 1 Validation of the method considering intra-day and inter-day precision

Concentration (μmol L^{-1})	Precision (%RSD)	
	Intra-day	Inter-day
10.0	1.48	1.76
1.0	2.56	2.87
0.1	4.94	5.48

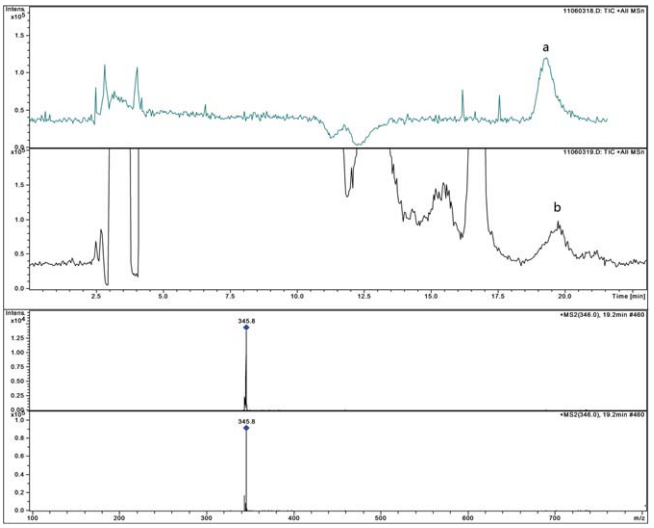


Fig. 4 The mass spectra of standard JA (a, green) and extraction of wintersweet (b, black) after derivatization. Mobile phase: 0.04 mol L^{-1} ammonium acetate buffer solution (pH 5.1) and acetonitrile (67 : 33, v/v). Flow rate: 0.80 mL min^{-1}.

results proved that this derivatization reaction was feasible and successful. Consequently, endogenous JA in wintersweet flowers was further detected by HPLC-ECD. The typical chromatograms are shown in Fig. 5. The content of JA in wintersweet flowers was 7.86 μg g^{-1} calculated from the peak area of JA–DA. This result was in good agreement with that by LC-UV in our group. However, the complicated matrix of the crude extracts of wintersweet flowers resulted in a relatively low recovery (66.2%).

In order to improve the recovery of this method, the standard addition method as an alternative was introduced into the analysis of endogenous JA in rice florets containing a rather lower concentration of JA than that in wintersweet flowers. Different concentrations of JA standard solution ranging from 4.0×10^{-7} mol L^{-1} to 1.0×10^{-5} mol L^{-1} were added into crude extracts of rice florets with the same volume, and the

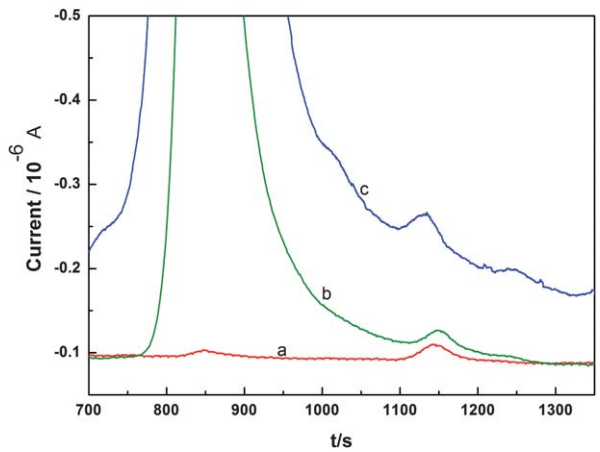


Fig. 5 Chromatograms of derivatized standard JA (curve a), extracts of wintersweet (curve b) and spiked wintersweet with standard JA (curve c). Mobile phase: 0.04 mol L^{-1} acetate buffer solution (pH 5.1) and acetonitrile (67 : 33, v/v). Flow rate: 0.80 mL min^{-1}. Applied potential: +0.9 V.

derivatization and analysis procedure did not change following the reported method for the determination of chloride in polluted water by Watkins.[23] Fig. 6 shows the linear relationship between the peak area of the JA–DA derivative and concentrations of added standard JA. The linear regression equation is Q (nA s) = 5.489 + 0.45c (μmol L^{-1}) (R = 0.9993, n = 6). The initial endogenous JA content in rice floret samples (C_0) can be obtained from the intercept of plot (peak area *vs.* C_{JA}) by extrapolation to the vertical axis at peak area = 0. The value of C_0 was derived to be 1.22 × 10^{-6} mol L^{-1} and endogenous JA in rice florets was calculated to be 308 ng g^{-1} which was similar to the result in our group using the LC-MS method. In addition, the recoveries of this method ranged from 88.60% to 93.83%, indicating that this method is feasible and can be successfully applied to real samples .

Experimental

Reagents and apparatus

Jasmonic acid (JA) was purchased from Sigma-Aldrich (USA) and was dissolved in acetonitrile to form 0.01 mol L^{-1} stock solution. The stock solution was stored in darkness at −4 °C and could be further diluted to desired concentrations by acetonitrile. A stock solution of 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC·HCl), obtained from Aladdin (Shanghai, China), was prepared by dissolving it in acetonitrile to form a concentration of 0.002 mol L^{-1}. Dopamine hydrochloride (3-hydroxytyramine hydrochloride, DA·HCl) from Fluka was dissolved in methanol at a concentration of 0.02 mol L^{-1}. Acetonitrile was of HPLC grade and other chemicals were analytical reagents, including all inorganic salts used to prepare the buffer solution. 0.22 mm Nylon filter membrane was bought from Shanghai Xingya Jinghua Materials Factory (Shanghai, China). All the chemicals were used without further purification and all the solutions were prepared with deionized water.

The HPLC separation was carried out on a Shimadzu LC-20AT system (Kyoto, Japan) equipped with a manual injector (20 μL) and a Sepax amethyst C18-P column (5 mm, 4.6 × 250 mm; Sepax Technologies, USA). The electrochemical analysis was performed on a CHI842B electrochemical analyzer with a thin layer flow cell (Shanghai Chenhua Instruments, Shanghai, China). A three-electrode system was employed: Ag/AgCl (saturated KCl) as the reference electrode, a stainless steel auxiliary block as the counter electrode, and a glassy carbon electrode (1 mm, GCE) as the working electrode.

For high performance liquid chromatography coupled with mass spectrometry (LC-MS) analysis, an Agilent 1100 HPLC system was coupled on-line to an ion-trap mass spectrometer (Agilent Corp., Waldbronn, Germany) equipped with an electrospray ionization (ESI) source. The separation was performed using a Sepax amethyst C18-P column (5 mm, 4.6 × 250 mm; Sepax Technologies, USA). The auto MS operation parameters were as follows: positive ion mode (ESI^+); nitrogen drying gas, 10 L min^{-1}; nebulizer, 40 psi; gas temperature: 350 °C; compound stability, 80%; mass range, 50–1000 m/z. Detection of the JA–DA derivative was performed in selected ion monitoring (SIM) mode with $(m/z)^+$ 346.

Sample extraction

The wintersweet flowers, from the campus of Wuhan University, were stored at −20 °C once picked. 10 g of the flower sample were soaked in acetonitrile for 6 h in an ultrasonic bath at room temperature, and then overnight at −20 °C. The extraction solution was evaporated to 3 mL the next day. Subsequently, the supernatant was collected after centrifugation at 10 000 rpm for 10 min for further derivatization reaction. The rice floret samples, obtained from Jiangxi Agricultural University (China), were frozen in liquid nitrogen once after collection and lyophilized. A portion of 3 g of rice floret samples was treated in the same way as the wintersweet flowers mentioned above.

Derivatization of JA

The use of EDC·HCl as condensing agent in the formation of peptide bonds of amino acids is a classic reaction. 10 μL of JA standard solution was added into an Agilent screw-thread neck vial, and 100 μL of EDC·HCl stock solution was then added to the vial to activate the carboxylic acid group of JA. The solution was agitated, followed by addition of 100 μL of the DA·HCl solution. The total final volume was set to 1 mL by acetonitrile and the reaction was undertaken at 60 °C for 12 h. The mixture was directly used for separation and detection.

Analytical procedure

The flow rate was set at 0.8 mL min^{-1}. A mobile phase consisting of acetonitrile–ammonium acetate buffer (pH 5.07; 40 mmol L^{-1}) (33 : 67, v/v) was used in this experiment. Prior to the experiment, both mobile phases were filtered through a 0.22 mm Nylon filter membrane and degassed in an ultrasonic bath for 30 min. The GCE was polished with alumina slurry to a mirror finish and rinsed with water. Before the analysis, the chromatographic system was equilibrated for over 30 min. Chromatographic amperometric i–t curves of the samples were

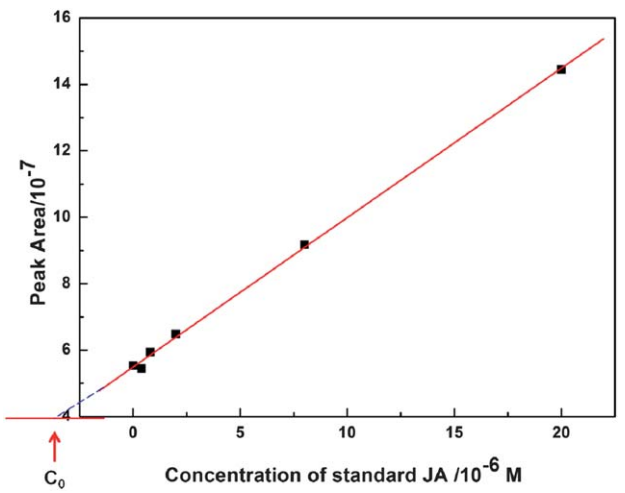


Fig. 6 Linear relationship between the peak area of the JA–DA derivative and the concentration of added standard JA in rice florets.

recorded at a working potential of 0.90 V. All experiments were performed at room temperature.

Conclusions

We have successfully solved two bottleneck problems in the development of LC-ECD methods for the analysis of endogenous JA, by the novel use of pre-column derivatization of JA with electroactive dopamine. The oxidation of the JA derivative with dopamine can be realized under mild conditions (pH 7.0 phosphate buffer or pH 5.1 acetate buffer solution), so that the supporting electrolyte can be used as the mobile phase of LC separation at relatively low potentials; below 0.6 V. The detection sensitivity is greatly improved by almost 3 orders from LOD 10^{-5} (ref. 20) to 5.0×10^{-8} mol L^{-1}. The developed method has been successfully applied in the determination of JA content in real samples, wintersweet flowers and rice florets. The results were consistent with those by LC-UV and LC-MS methods in our group, indicating that this novel pre-column electrochemical derivatization method is indeed feasible. Further research on the improvement of recovery is in progress in our group.

Acknowledgements

This work was supported by the National Scientific Foundation of China (Grant no.: 90817103, 30973672, 20775055), Doctoral Fund of Ministry of Education of China (no. 20110141110024), Hubei Provincial Scientific Foundation (no. 2011CDB475) and the Fundamental Research Funds for the Central Universities.

Notes and references

1 D. G. Aldrige, S. Galt and D. Giles, *J. Chem. Soc. C*, 1971, 1623–1627.
2 R. Rajiv, M. Otto and S. Gunther, *Physiol. Plant.*, 1994, **90**(3), 548–552.
3 M. U. Rita, R. Marcela and C. M. Gustava, *Physiol. Plant.*, 2002, **115**, 417–427.
4 K. Satoru and F. Kayoka, *Sci. Hortic.*, 2001, **91**, 275–288.
5 Y. Ishii, H. Kiyota, S. Sakai and Y. Honma, *Leukemia*, 2004, **18**, 1413–1419.
6 J. H. Kim, S. Y. Lee, S. Y. Oh, S. I. Han, H. G. Park, M. A. Yoo and H. S. Kang, *Oncol. Rep.*, 2004, **12**, 1233–1238.
7 D. Samaila, D. E. Ezekwudo and J. A. Yimam, *Trans. Integr. Biomed. Inform. Enabling Tech. Symp. J.*, 2004, 34–42.
8 O. Fingrut and E. Flescher, *Leukemia*, 2002, **16**, 608–616.
9 Y. H. Han, Y. P. Liao and H. W. Liu, *Chin. J. Anal. Chem.*, 2009, **10**, D142.
10 E. Juergen, A. S. Eric, T. A. Hans, J. C. Yasmin, H. Juan and H. T. James, *Anal. Biochem.*, 2003, **312**(2), 242–250.
11 F. J. Zhang, Y. J. Jin, X. Y. Xu, R. C. Lu and H. J. Chen, *Phytochem. Anal.*, 2008, **19**, 560–567.
12 R. Krarnell, G. Schneider and O. Mlersch, *Phytochem. Anal.*, 1996, **7**, 209–212.
13 G. Schneider, R. Krarnell and C. Bruckner, *J. Chromatogr., A*, 1999, **847**, 103–107.
14 J. M. Anderson, *J. Chromatogr., A*, 1985, **330**, 347–355.
15 J. M. Anderson, *Anal. Biochem.*, 1986, **152**, 146–153.
16 Y. H. Han, Z. G. Zhou, Y. Bai, H. W. Liu, H. L. Wu and R. Lei, *J. Chromatogr., A*, 2012, **1235**, 125–131.
17 X. Q. Pan, W. Ruth and X. M. Wang, *Phytochemistry*, 2008, **69**, 1773–1781.
18 N. Hideaki, Y. Hisakazu, Y. Isomaro, S. Hideharu, M. Noboru, Y. Teruhiko and S. Hiroh, *Plant Cell Physiol.*, 1992, **33**, 1225–1231.
19 A. X. Deng, W. M. Tan, S. P. He, *et al.*, *J. Integr. Plant Biol.*, 2008, **50**, 1046–1052.
20 Y. Q. Liao, F. Wang and Z. L. Chen, *Chin. Sci. Bull.*, 2010, **55**, 2225–2230.
21 W. P. Zhang and Z. L. Chen, *Anal. Lett.*, 2013, **46**, 74–86.
22 J. Chen, Q. H. Chen and Z. L. Chen, *Analyst*, 2012, **137**, 5436–5440.
23 P. J. Watkins, *Electroanalysis*, 1997, **9**, 85–86.