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# Ultrasensitive Determination of Jasmonic Acid in Plant Tissues Using High-Performance Liquid Chromatography with Fluorescence Detection

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Supporting Information

**ABSTRACT:** An ultrasensitive and selective high-performance liquid chromatographic method for the volatile signaling hormone, jasmonic acid, has been developed based on precolumn derivatization with 1,3,5,7-tetramethyl-8-aminozide-difluoroboradiaza-*s*-indacene (BODIPY-aminozide). The derivatization reaction was carried out at 60 °C for 30 min in the presence of phosphoric acid. The formed jasmonic acid derivative was eluted using a mobile phase of methanol/pH 6.50 ammonium formate buffer/tetrahydrofuran (67:30:3, v/v/v) in 10 min on a $C_{18}$ column and detected with fluorescence detection at excitation and emission wavelengths of 495 and 505 nm, respectively. The detection limit (signal-to-noise ratio = 4) reached $1.14 \times 10^{-10}$ M or 2.29 fmol per injection (20 $\mu$L), which is the lowest of the existing methods. The proposed method has been successfully applied to the direct determination of trace jasmonic acid in the crude extracts of soybean leaves from soybean mosaic virus-infected and normal plants with recoveries of 95−104%.

**KEYWORDS:** *1,3,5,7-tetramethyl-8-aminozide-difluoroboradiaza-s-indacene, jasmonic acid, fluorescent labeling, high-performance liquid chromatography*

## INTRODUCTION

Jasmonic acid, a representative of jasmonates,[1] plays an important role in a variety of plant physiological processes such as fruit ripening, plant growth, development, and senescence.[2−4] It also 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.[5−7] Some investigations indicate that the roles of jasmonic acid in plants are concentration-dependent.[8,9] Thus, the determination of endogenous jasmonic acid in plant tissues is of great significance for the study of the physiological function and action mechanism of jasmonic acid. However, the concentration of jasmonic acid in plant tissues is between 10 and 1500 ng/g depending on the plant developmental stage as well as environmental stimuli and physiological conditions.[10] Considering the extremely low level of jasmonic acid existing in complex plant samples, a sensitive and selective method for the determination of jasmonic acid is needed all the while.

Some analytical methods have been reported for the quantitative determination of jasmonic acid, including radioimmunoassay,[11] enzyme-linked immunosorbent assay (ELISA),[12,13] gas chromatography−mass spectrometry (GC/MS),[14−18] liquid chromatography−mass spectrometry (LC/MS) or liquid chromatography−tandem mass spectrometry (LC/MS/MS),[19,20] high-performance liquid chromatography coupled with fluorescence detection (HPLC-FD),[10,21,22] capillary electrophoresis−mass spectrometry (CE-MS),[23] and capillary electrophoresis with laser-induced fluorescence detection (CE-LIF).[24] It has been reported that GC/MS and ELISA are well suitable for the assay of large numbers of samples and exhibit good selectivity for jasmonic acid.[13,25] However, these methods have some inherent problems. For example, the ELISA method is quite simple to operate, but it exhibits cross-reactivity of structurally related compounds presented in the same sample. GC/MS needs a complicated and intensive purification protocol, which are time-consuming and tedious.[14,26,27]

HPLC and CE combined with fluorescence detection both show high sensitivity and selectivity in the determination of jasmonic acid.[21,22,24] Because of the lack of a strong chromophores or fluorophores in the chemical structure of jasmonic acid, fluorescent labeling becomes a necessary procedure before the detection. The keto or carboxyl group in the chemical structure of jasmonic acid (Figure 1) can be derivatized; therefore, two derivatization protocols have been reported. One is based on hydrazine reagents for the derivatization of carbonyl group;[21] the other is based on the carboxyl-reactive reagents.[22,24] L-Dimethylaminonaphthalene-5-sulfonylohydrazide (dansyl hydrazine) is the first fluorescent labeling reagent used for the quantitation of jasmonic acid by means of reaction with carbonyl group in HPLC.[21] However, up to 20% of the jasmonic acid will convert to the

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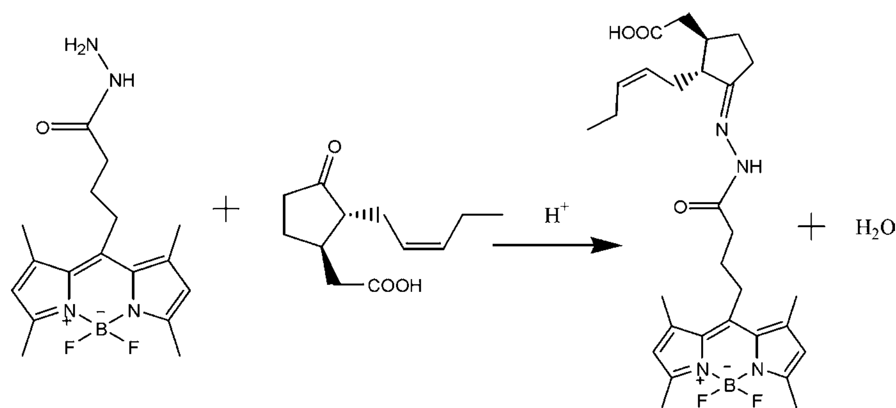


**Figure 1.** Derivatization reaction of BODIPY-aminozide with jasmonic acid.

corresponding ester during the derivatization. Although good selectivity and reproducibility can been obtained for the HPLC determination of jasmonic acid using 9-anthryldiazomethane,[22] 9-anthryldiazomethane is not stable at room temperature and must be synthesized in situ, which creates problems in the separation of jasmonic acid from the fluorescent impurities. Another carboxyl-reactive reagent used in the determination of jasmonic acid with CE-LIF is 5-bromomethylfluorescein, and the detection limit of jasmonic acid reaches $10^{-17}$ mol per injection in this case.[24] The limitations of 5-bromomethylfluorescein are ascribed to the rigid derivatization conditions, such as high derivatization limit (0.5 $\mu$M), expensive catalyst (18-crown-6), and anhydrous conditions.

In this work, 1,3,5,7-tetramethyl-8-aminozide-difluoroboradiaza-*s*-indacene (BODIPY-aminozide), which was synthesized in our laboratory as a carbonyl-reactive reagent recently,[28] has been used to address the problems forementioned. As a BODIPY analogue, it has much better properties including the strong fluorescence, excellent stability, and independence of solvent and pH in contrast to the case of fluorescein.[29] The reaction group of BODIPY-aminozide is aminozide, which can label aldehyde or carboxyl selectively by using different catalysts such as acid or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC).[30] Using BODIPY-aminozide as a precolumn derivatizing reagent, an HPLC-FD method has been developed for the highly sensitive determination of jasmonic acid in plant samples. The proposed method has advantages of relatively mild derivatization conditions, rather short derivatization time and separation time, high selectivity, and the lowest detection limit as compared with the existing methods.

## ■ MATERIALS AND METHODS

**Reagents.** 1,3,5,7-Tetramethyl-8-aminozide-difluoroboradiaza-*s*-indacene was synthesized in our laboratory. Methanol and acetonitrile (Shanghai Chemical Reagent Co., Shanghai, China) were of HPLC grade. The phosphorous acid solution (catalyst) for the derivatization was prepared by diluting phosphorous acid with double-distilled water. Water was purified on a Milli-Q system (Millipore, Bedford, MA). Unless otherwise specified, all reagents were of analytical grade and used without further purification.

The BODIPY-aminozide solution ($1.0 \times 10^{-3}$ M) was prepared by dissolving 3.5 mg of BODIPY-aminozide in 10 mL of acetonitrile. Jasmonic acid (Sigma, St. Louis, MO) stock solution ($1.00 \times 10^{-4}$ M) was prepared in acetonitrile. Standard solutions of jasmonic acid were prepared by further dilution of jasmonic acid stock solution with acetonitrile to get the standard curve and to spike plant samples for determination of the recovery. When not in use, all solutions were stored at 4 °C. BODIPY-aminozide solution could be used for 4 weeks.

**Apparatus.** The LC-10A series HPLC system (Shimadzu, Tokyo, Japan) was used in the experiments. It comprised the following modules: a Shimadzu LC-10 AD dual-pump, an RF-10AXL fluorescence detector (Shimadzu), a column oven (Shimadzu), a 2010 chromatography Chemstation (Zhejiang University, Hangzhou, China), and a manual injection (20 $\mu$L). Fluorescence detection wavelengths were set at $\lambda_{ex}/\lambda_{em}$ = 495/505 nm. Chromatographic separation was accomplished on a 250 mm × 4.6 mm i.d., 5 $\mu$m RP-18 reverse phase column (Kromasil, Bohus, Sweden) at room temperature. The pH value of solution was measured using a Mettler Toledo Delta 320 m (Mettler-Toledo, Shanghai, China).

**Samples Preparation.** Soybean leaves were collected from soybean mosaic virus-infected and untreated plants and prepared according to previous publications,[19,24] with minor modifications. Fresh soybean leaves were washed with deionized water and dried gently with absorbent paper by removing water on the surface and then ground to a fine powder in the presence of liquid nitrogen and stored at −80 °C until extraction. Approximately 500 mg of sample powder was homogenized in 2.5 mL of cold acetonitrile by a 2 min sonication at 4 °C. After ultrasonication at 4 °C for 10 min and vortexing for 10 min, the mixture was centrifuged for 5 min at 4 °C and 4500 rpm, and the supernatant containing jasmonic acid was collected. The residues were repeatedly extracted using 0.5 mL of acetonitrile for three times. All of the supernatant was combined and filtered with a 0.45 $\mu$m filter and then stored at 4 °C for use.

**Derivatization of Jasmonic Acid.** Ninety microliters of BODIPY-aminozide solution ($1.0 \times 10^{-3}$ M), 10 $\mu$L of phosphorous acid (0.1 M), and 900 $\mu$L of jasmonic acid standard solution at different concentrations were successively added into a 1.0 mL vial. The mixture was diluted to the mark with acetonitrile and kept at 60 °C for 0.5 h. After the derivatization (Figure 1) was completed, the solution was cooled to room temperature. An aliquot (20 $\mu$L) of the mixture was injected directly into the chromatograph system. The reagent blanks without jasmonic acid were also treated similarly. Derivatization of real samples was carried out in the same way using 900 $\mu$L of plant extract.

## ■ RESULTS AND DISCUSSION

**Optimization of Derivatization Conditions.** To establish the optimal conditions for the lowest detection of jasmonic acid, the effects of parameters on the derivatization reaction of BODIPY-aminozide with jasmonic acid were investigated. The reaction temperature and time are always important factors on the derivatization yield. Varying the reaction time from 15 min to 2.5 h and the reaction temperature in the range 25−60 °C, the effect was studied. The results indicated that the highest and constant detector response appears at 60 °C, and the reaction time to the plateau is shortest. Further experiment

shows that a reaction temperature higher than 60 °C could not increase the peak area of jasmonic acid, might lead to slight decomposition of BODIPY-aminozide, and finally extend the separation time because of the interference of peaks from decomposition products. Therefore, a derivatization at 60 °C for 30 min was employed.

Generally, excess of labeling reagents should be used for the quantitative analysis of jasmonic acid to ensure the derivatization efficiency and reproducibility. Therefore, increasing the molar ratio of BODIPY-aminozide to jasmonic acid would be favorable to improve the yield of BODIPY-aminozide-jasmonic acid derivative. The derivative yield was investigated with the molar ratio in the range of 10:1 to 400:1. The highest and most constant detector response was obtained with the molar ratios of BODIPY-aminozide to jasmonic acid above 200:1; therefore, a molar ratio of 200:1 was selected.

For carboxyl-reactive labeling reagents, the water content in reaction media affects the derivatization yields severely.[22,24] The derivatization reaction is even inhibited by water when using 9-anthryldiazomethane as a labeling reagent.[22] Correspondingly, the plant extracts are generally evaporated to dryness under vacuum or under a $N_2$ stream to remove water. Meanwhile, this procedure will cause the loss of volatile compositions including jasmonic acid. In our work, the reaction of BODIPY-aminozide with carbonyl group is an addition reaction, which releases water (Figure 1), and the water content of reaction solutions might influence hydrazone formation also.[31] Therefore, the influence of water on the derivatization yield has been examined. It is found that water content from 1 to 20% has no significant effects on the derivatization yields; that is, anhydrous condition is not necessary. As a result, the evaporation to dryness of plant tissue extracts, in which water is less than 10%, is needless.

**Separation of BODIPY-Aminozide-Jasmonic Acid Derivative.** The preliminary separation of BODIPY-aminozide-jasmonic acid derivative and the reagent was carried out in isocratic elution mode at a flow rate of 1.0 mL/min on $C_{18}$ column with the fluorescence detection at $\lambda_{ex}/\lambda_{em}$ = 495/505 nm at room temperature. On the basis of this, the optimization of the mobile phase was performed. The increasing methanol concentration in the mobile phase can shorten the retention times of both reagent and BODIPY-aminozide-jasmonic acid derivative. When the composition of methanol in the mobile phase is less than 70% (v/v), the reagent and BODIPY-aminozide-jasmonic acid derivative can be well separated at the baseline, but the peak of jasmonic acid derivative is broad. To sharpen the peak shape and shorten retention time, tetrahydrofuran (THF) was added to the mobile phase. When the THF content is 3% (v/v), the improvement is most conspicuous. Consequently, 3% (v/v) THF was added in the mobile phase.

The influence of pH of ammonium-formate buffer in the mobile phase on the separation of the reagent and jasmonic acid derivative was not obvious in the pH range from 2.50 to 8.50. Increasing pH value, the retention time of jasmonic acid derivative is slightly shortened for the ionizability of the carboxyl on the jasmonic acid derivative would rise in the media with high pH value. The operation at pH < 6.00 results in tailing of the reagent. The use of pH 6.50 ammonium-formate buffer can effectively prevent the tailing of the regent and improve the separation of jasmonic acid and the reagent. The buffer concentration in the range of 40–60 mM results in good reproducibility of the separation and 50 mM, pH 6.50, ammonium-formate buffer was used.

On the basis of the above investigations, the mobile phase consisting of a mixture of methanol–buffer (pH 6.50)–THF = 67:30:3 (v/v/v) was used. Typical chromatograms of reagent blank and standard solution of jasmonic acid are presented in Figure 2A.

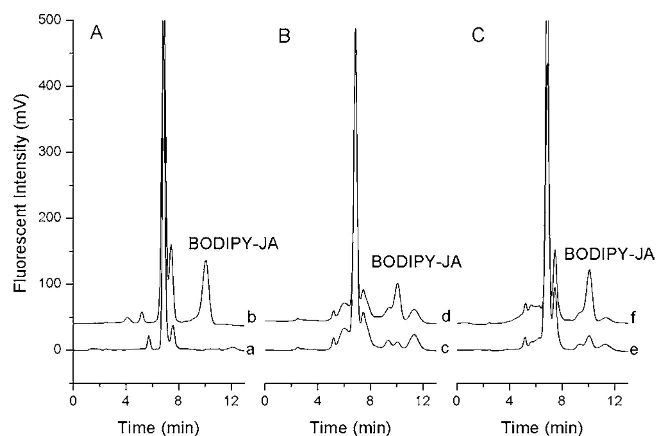


**Figure 2.** Chromatograms of standard jasmonic acid (A), healthy soybean leaf (B), and pathogen-infested soybean leaf (C). (a) Blank, (b) standard jasmonic acid, (c) healthy soybean leaf, (d) healthy soybean leaf spiked with 20 nM standard jasmonic acid, (e) pathogen-infested soybean leaf, and (f) pathogen-infested soybean leaf spiked with 20 nM standard jasmonic acid. Peak: (1) BODIPY-jasmonic acid.

**Selectivity.** As a carbonyl-reactive reagent, BODIPY-aminozide used in this research can also react with other coexisted carbonyl-containing compounds, like aliphatic and aromatic aldehydes or ketones, jasmonic acid conjugates, and phytohormones-containing ketone groups. After systematic experiments, it was found that although aliphatic and aromatic aldehyde/ketones could react with BODIPY-aminozide easily, their derivatives are eluted behind jasmonic acid derivative even for formaldehyde derivative because of the thoroughly different structure between jasmonic acid and them. Some ketone-containing phytohormones such as abscisic acid and methyl jasmonate can also react with BODIPY-aminozide and may interfere with the elution of jasmonic acid. However, they exhibit rather low reactivity to BODIPY-aminozide under the present derivatization conditions, and the coexistence of them at $1.0 \times 10^{-4}$ M level is allowed. By raising the reaction temperature to 80 °C and prolonging the reaction time to 2–3 h, they can be derivatized with BODIPY-aminozide at a concentration of 10 nM. The reason for this phenomenon is still under investigation and will be studied systematically in the near future.

**Linear Range, Reproducibility, and Detection Limit.** The quantitative analysis of jasmonic acid was performed under the optimum derivatization and separation conditions mentioned above by derivatizing different amount of jasmonic acid standard solution from $5.0 \times 10^{-10}$ to $5.0 \times 10^{-7}$ M. The linearity was evaluated by plotting the peak area to jasmonic acid concentration. The linear regression equation for jasmonic acid was calculated as $Y = 1.78 \times 10^8 X - 1.26 \times 10^4$ ($R^2$ = 0.9987), in which $X$ is jasmonic acid concentration ($\mu$M) and $Y$ is peak area. The detection limit for jasmonic acid was determined by diluting the jasmonic acid standard derivatization solution and found to be $1.14 \times 10^{-10}$ M at a signal-to-

noise ratio of 3, and the mass detection limit of jasmonic acid was 2.29 fmol per injection. The precision of the proposed method was tested using six repeated derivatization reactions of standard solution mixtures of jasmonic acid and sample solution. The accuracy of the method was examined using standard addition procedure. Four series at different concentration levels of jasmonic acid were prepared by adding known amounts of jasmonic acid standard substance to sample solution. The intra- and interday precision and accuracy of the proposed method were evaluated in terms of relative standard deviations (RSDs) of the peak areas, respectively. High precision was observed with RSDs less than 3.0%.

**Determination of Jasmonic Acid in Plant Samples.** The new method described here has been applied to the determination of jasmonic acid in the acetonitrile extracts of soybean mosaic virus-infected and untreated soybean leaves. The recovery was estimated by spiking a known amount of jasmonic acid to plant samples. The chromatograms of the soybean leaf samples were shown in Figure 2B,C, and the analytical results are listed in Table 1. It was found that the jasmonic acid levels increased rapidly in soybean mosaic virus-infected samples and were about three times higher than those in the normal. These results suggest that the present method is suitable to the measurement of trace jasmonic acid in plant samples.

**Table 1. Analytical Results of Jasmonic Acid in Soybean Leaf Extracts**

| sample | jasmonic acid concn (nM)[a] added | jasmonic acid concn (nM)[a] found | RSD (%) | recovery (%) |
|---|---|---|---|---|
| extract of untreated soybean leaves | 0 | 2.22 | 2.74 | |
| | 5.00 | 7.06 | 2.03 | 96.8 |
| | 10.0 | 11.7 | 1.97 | 94.8 |
| | 20.0 | 23.0 | 3.63 | 103.9 |
| extract of mosaic virus-infected soybean leaves | 0 | 6.25 | 2.88 | |
| | 5.00 | 11.0 | 2.36 | 95.0 |
| | 10.0 | 16.5 | 2.18 | 102.5 |
| | 20.0 | 26.6 | 1.43 | 101.8 |

[a]Mean ($n$ = 6).

**Comparison of the Proposed Method with Some Typical Analytical Methods.** To evaluate the proposed method further, a thorough comparison of the present method with typical analytical methods reported previously is performed. The results indicate that the present method has the highest sensitivity, good selectivity, and simplest sample preparation among the available methods. In addition, it is worthy to note that the present method also has advantages of relatively mild derivatization conditions, rather short derivatization time (30 min), and separation time (10 min).

In summary, the method presented here provides a new approach for the ultra sensitive and selective quantitation of jasmonic acid in plant samples. Our research demonstrates that this HPLC-FD with BODIPY-aminozide labeling method has powerful potential in monitoring trace jasmonic acid involved in complex physiological and biochemical processes in various plant samples, such as tissues and cells.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Effects of reaction temperature and reaction time on the derivatization yield (Figure 1S), effect of molar ratio of BODIPY-aminozide to jasmonic acid on the derivatization yield (Figure 2S), precision and accuracy for the analysis of jasmonic acid (Table S1), and comparison of the present method with some typical analytical methods (Table S2). This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

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### Notes

The authors declare no competing financial interest.

## ■ REFERENCES

(1) Creelman, R. A.; Mullet, J. E. Biosynthesis and action of jasmonates in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1997**, *48*, 355−381.

(2) Meyer, A.; Miersch, O.; Biittner, C.; Dathe, W.; Sernbdner, G. Occurrence of the plant growth regulator jasmonic acid in plants. *J. Plant Growth Regul.* **1984**, *3*, 1−8.

(3) Creelman, R. A.; Mullet, J. E. Oligosaccharins, brassinolides, and jasmonates: Nontraditional regulators of plant growth, development, and gene expression. *Plant Cell* **1997**, *9*, 1211−1223.

(4) Wasternack, C. Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Ann. Bot.* **2007**, *100*, 681−697.

(5) Wasternack, C.; Parthier, B. Jasmonate-signalled plant gene expression. *Trends Plant Sci.* **1997**, *2*, 302−307.

(6) Wasternack, C.; Stenzel, I.; Hause, B.; Hause, G.; Kutter, C.; Maucher, H.; Neumerkel, J.; Feussner, I.; Miersch, O. J. The wound response in tomato-role of jasmonic acid. *Plant Physiol.* **2006**, *163*, 297−306.

(7) Brossa, B.; Lopez-Carbonell, M.; Jubany-Marı, T.; Alegre, L. Interplay between abscisic acid and jasmonic acid and its role in water-oxidative stress in wild-type, ABA-deficient, JA-deficient, and ascorbate-deficient *Arabidopsis* plants. *J. Plant Growth Regul.* **2011**, *30*, 322−333.

(8) Anderson, J. M. Jasmonic acid-dependent increases in the level of specific polypeptides in soybean suspension cultures and seedlings. *J. Plant Growth Regul.* **1988**, *7*, 203−211.

(9) Ludwig-Müller, J.; Bennett, R.; García-Garrido, J.; Piché, Y.; Vierheilig., H. Reduced arbuscular mycorrhizal root colonization in *Tropaeolum majus* and *Carica papaya* after jasmonic acid application can not be attributed to increased glucosinolate levels. *J. Plant Physiol.* **2002**, *159*, 517−523.

(10) Wilbert, S. M.; Ericsson, L. H.; Gordon, M. P. Quantification of jasmonic acid, methyl jasmonate, and salicylic acid in plants by capillary liquid chromatography electrospray tandem mass spectrometry. *Anal. Biochem.* **1998**, *257*, 186−194.

(11) Knoêfel, H. D.; Bruêckner, C.; Kramell, R.; Sembdner, G.; Schreiber, K. Radioimmunoassay for the natural plant growth regulator (−)-jasmonic acid. *Biochem. Physiol. Pflanz.* **1990**, *186*, 387−394.

(12) Albrecht, T.; Kehlen, A.; Stahl, K.; Knöfel, H. D.; Sembdner, G.; Weiler, E. W. Quantification of rapid, transient increases in jasmonic acid in wounded plants using a monoclonal antibody. *Planta* **1993**, *191*, 86−94.

(13) Zhang, F. J.; Jin, Y. J.; Xu, X. Y.; Lu, R. C.; Chen, H. J. Study on the extraction, purification and quantification of jasmonic acid, abscisic acid and indole-3-acetic acid in plants. *Phytochem. Anal.* **2008**, *19*, 560−567.

(14) Engelberth, J.; Schmelz, E. A.; Alborn, H. T.; Cardoza, Y. J.; Huang, J.; Tumlinson, J. H. Simultaneous quantification of jasmonic acid and salicylic acid in plants by vapor-phase extraction and gas chromatography-chemical ionization-mass spectrometry. *Anal. Biochem.* **2003**, *312*, 242−250.

(15) Baldwin, I. T.; Zhang, Z. P.; Diab, N.; Ohnmeiss, T. E.; McCloud, E. S. Quantification, correlations and manipulations of wound-induced changes in jasmonic acid and nicotine in *Nicotiana sylvestris*. *Planta* **1997**, *201*, 397−404.

(16) Birkemeyer, C.; Kolasa, A.; Kopka, J. Comprehensive chemical derivatization for gas chromatography-mass spectrometry-based multi-targeted profiling of the major phytohormones. *J. Chromatogr., A* **2003**, *993*, 89−102.

(17) Koo, Y. J.; Yoon, E.; Song, J. T.; Seo, H. S.; Kim, J. H.; Lee, Y. H.; Lee, Y. W.; Lee, J. S.; Cheong, J. J.; Choi, Y. D. An advanced method for the determination of carboxyl methyl esterase activity using gas chromatography-chemical ionization-mass spectrometry. *J. Chromatogr., B* **2008**, *863*, 80−87.

(18) Nojiri, H.; Yamane, H.; Seto, H.; Yamaguchi, I.; Murofushi, N.; Yoshihara, T.; Shibaoka, H. Qualitative and quantitative analysis of endogenous jasmonic acid in bulbing and non-bulbing onion plants. *Plant Cell Physiol.* **1992**, *33*, 1225−1231.

(19) Pan, X. Q.; Welti, R.; Wang, X. M. Simultaneous quantification of major phytohormones and related compounds in crude plant extracts by liquid chromatography-electrospray tandem mass spectrometry. *Phytochemistry* **2008**, *69*, 1773−1781.

(20) Segarra, G.; Jáuregui, O.; Casanova, E.; Trillas, I. Simultaneous quantitative LC-ESI-MS/MS analyses of salicylic acid and jasmonic acid in crude extracts of *Cucumis sativus* under biotic stress. *Phytochemistry* **2006**, *67*, 395−401.

(21) Anderson, J. M. Simultaneous determination of abscisic acid and jasmonic acid in plant extracts using high-performance liquid chromatography. *J. Chromatogr.* **1985**, *330*, 347−355.

(22) Kristl, J.; Veber, M.; Krajničič, B.; Orešnik, K. Determination of jasmonic acid in *Lemna minor* (L.) by liquid chromatography with fluorescence detection. *Anal. Bioanal. Chem.* **2005**, *383*, 886−893.

(23) Han, Y.; Bai, Y.; Xiao, Y.; Du, F.; Liang, Y.; Tan, Z.; Zhao, M.; Liu., H. Simultaneous discrimination of jasmonic acid stereoisomers by CE-QTOF-MS employing the partial filling technique. *Electrophoresis* **2011**, *32*, 2693−2699.

(24) Zhang, Z. L.; Liu, X.; Li, D. F.; Lu, Y. T. Determination of jasmonic acid in bark extracts from *Hevea brasiliensis* by capillary electrophoresis with laser-induced fluorescence detection. *Anal. Bioanal. Chem.* **2005**, *382*, 1616−1619.

(25) Pan, X. Q.; Wang, X. M. Profiling of plant hormones by mass spectrometry. *J. Chromatogr., B* **2009**, *877*, 2806−2813.

(26) Müller, A.; Düchting, P.; Weiler, E. W. A multiplex GC-MS/MS technique for the sensitive and quantitative single-run analysis of acidic phytohormones and related compounds, and its application to *Arabidopsis thaliana*. *Planta* **2002**, *216*, 44−56.

(27) Zadra, C.; Borgogni, A.; Marucchini, C. Quantification of jasmonic acid by SPME in tomato plants stressed by ozone. *J. Agric. Food Chem.* **2006**, *54*, 9317−9321.

(28) Xiong, X. J.; Wang, H.; Rao, W. B.; Guo, X. F.; Zhang, H. S. 1,3,5,7-Tetramethyl-8-aminozide-difluoroboradiaza-s-indacene as a new fluorescent labeling reagent for the determination of aliphatic aldehydes in serum with high performance liquid chromatography. *J. Chromatogr., A* **2010**, *1217*, 49−56.

(29) Ulrich, G.; Ziessel, R.; Harriman, A. The chemistry of fluorescent bodipy dyes: versatility unsurpassed. *Angew. Chem., Int. Ed.* **2008**, *47*, 1184−1201.

(30) Guo, X. F.; Zhou, Y.; Tu, F. Q.; Xiong, X. J.; Wang, H.; Zhang, H, S. Determination of phytohormones in plant samples based on the precolumn fluorescent derivatization with 1,3,5,7-tetramethyl-8-aminozide-difluoroboradiaza-s-indacene by HPLC for routine use. *J. Sep. Sci.* **2011**, *34*, 789−795.

(31) Binding, N.; Kläning, H.; Karst, U.; Pötter, W.; Czeschinski1, P. A.; Witting, U. Analytical reliability of carbonyl compound determination using 1,5-dansylhydrazine-derivatization. *Fresenius' J. Anal. Chem.* **1998**, *362*, 270−273.