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Determination of Chiral Jasmonates in Flowers by GC/MS after Monolithic Material Sorptive Extraction

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Supporting Information

ABSTRACT: A GC/MS method with monolithic material sorptive extraction (MMSE) pretreatment was developed to determine contents of the enantiomers of jasmonic acid and methyl jasmonate in flowers. To optimize MMSE extraction, several MMSE parameters were investigated, including extraction temperature, extraction time, and extraction solvent. Under the optimal conditions, extraction efficiency was good. Using the selected-ion monitoring mode, the limit of detection (LOD, S/N = 3) for methyl jasmonates was 0.257 ng/mL. The limit of quantitation (LOQ, S/N = 10) was 0.856 ng/mL. The linearity range was 1−100 ng/mL. The average recovery of methyl jasmonate at lower concentration was 116.8% (2 ng/mL). The relative standard deviation of methyl jasmonate contents determined within the linear range of detection was less than or equal to 15% of the mean determined level. The proposed method is rapid, sensitive, and competently applied to the determination of jasmonic acid and methyl jasmonate enantiomers in flowers.

KEYWORDS: *jasmonic acids, chiral separation, gas chromatography/mass spectrometry, monolithic material sorptive extraction, endogenous jasmonates*

1. INTRODUCTION

Both jasmonic acid and methyl jasmonate are important plant hormones that control signal transduction and regulate growth. They have been implicated as playing key roles in inducing herbivore-specific defense responses, and appear to be also involved in some pathogen-induced defense responses.[1] However, analysis of jasmonic acid and methyl jasmonate has been complicated by the low concentrations in which they are found in plant extracts, which are rich in substances that interfere with their detection.[2]

The methyl jasmonate molecule possesses two chiral centers at C-3 and C-7, each of which can have either the *R* or *S* absolute configuration. As a consequence, this compound can exist as four possible stereoisomers: the (−)-(3*R*,7*R*) and the (+)-(3*S*,7*S*) forms (which are named (−)- and (+)-methyl jasmonate, respectively) and the (−)-(3*S*,7*R*)- and its mirror image, the (+)-(3*R*,7*S*)-isomer (commonly known as (−)- and (+)-methyl epi-jasmonate, respectively (Figure 1)).[3] Enantiomers of methyl jasmonate show different biological properties: whereas (+)-epi methyl jasmonate appears to be the main contributor to the typical jasmine odor,[4] (−)-methyl jasmonate is the most active of the four stereoisomers in regulating the growth of rice seedlings.[5]

Qualitative and quantitative analyses of jasmonate have been performed using a variety of methods, including radioimmunoassay (RIA),[6] enzyme-linked immunosorbent assay (ELISA),[7−12] LC/MS,[13] GC/MS,[14] LC/MS/MS,[15] and GC/MS/MS.[16] However, quantification using RIA and ELISA can be misleading due to the complex matrix of the plant tissue extracts. Approaches involving LC/MS lack the high sensitivity. Given those approaches that use tandem mass spectrometers, such as GC/MS/MS and LC/MS/MS, are not widely used, GC/MS has thus emerged as the most popular method, owing to its cost-effectiveness and high sensitivity. As reported previously,[17] nonvolatile jasmonic acid should be converted into its volatile derivative methyl jasmonate prior to GC/MS analysis through methylation with diazomethane.

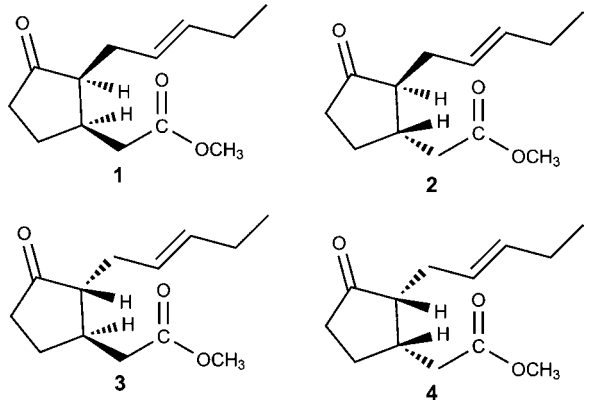

Figure 1. Chemical structures of the four stereoisomers of methyl jasmonate: **1**, (+)-(3*R*,7*S*)-methyl epi-jasmonate; **2**, (+)-(3*S*,7*S*)-methyl jasmonate; **3**, (−)-(3*R*,7*R*)-methyl jasmonate; **4**, (−)-(3*S*,7*R*)-methyl epi-jasmonate

Solid-phase extraction (SPE), a conventional sampling and concentration technique, uses a fused silica fiber coated with a polymer sorbent to concentrate analytes by adsorption and/or

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absorption.[18,19] Approaches that use SPE are widely used to analyze levels of plant hormones owing to their simplicity, lack of dependence on the solvent used, and amenability to convenient automation. Weber et al.[20] used C18-SepPak SPE cartridges to extract jasmonic acid. When Meyer et al.[21] employed a 100-μm polydimethylsioxane (PDMS) fiber as an extraction medium for headspace-SPME sampling, the limit of detection (LOD) of methyl jasmonate was 1.5 ng/g. Ruiz del Castillo[3] also used a PDMS-coated SPME fiber to extract methyl jasmonate. All of the approaches mentioned above involved sticks or stirrer bars coated in silicon polymer to a thickness of several micrometers using chemical bonding. However, the disadvantages of using a small surface area and thin coatings based on their low extraction capacity will lead to poor recovery rates and the need for a long sampling time.

In recent years, we have become interested in the development of analytical methods for endogenous jasmonic acids in plant. We have successfully developed simultaneous separation of jasmonic acid conjugates with amino acids by micellar electrokinetic chromatography[22] and a new method for enhancement of mass spectrometric and electrochemical detection sensitivity.[23,24] In this article, an MMSE technique that involves the use of the MonoTrap adsorbent combined with GC/MS analysis was developed to determine levels of jasmonic acid and its methyl ester in flowers. Monolith material is a polymer with porous structure; it was prepared by in situ polymerization. Minakuchi et al. reported the fabrication and evaluation of monolithic silica columns (silica rods) prepared via a so-called sol−gel process based on the hydrolytic polycondensation of alkoxysilanes.[25−27] MonoTrap is a collector developed using a completely new technique. It has a porous silica surface, which extends the surface area, and contains activated carbon, which enables adsorption of the target analyte(s). When a sample passes through the through-pores in a monolithic structure, the sample is trapped by ODS groups chemically bonded to the surface of the silica structure or by activated carbon present inside and outside the structure. Likewise, when extracting the solvent, solvent enters the through-pores and remains in contact with the entire surface to create speedy desorption of the sample. The device can therefore be used for highly sensitive analysis with rapid and effective analyte recovery. After MMSE, the extract can then be directly injected into GC/MS system.

2. MATERIALS AND METHODS

2.1. Reagents and Materials. Jasmonic acid standard solution (purity >98%) and methyl jasmonate standard solution (purity >98%) were purchased from Tokyo Chemical Industry (Tokyo, Japan), and (trimethylsilyl)diazomethane (2.0 M in hexane) was purchased from Anpel Scientific Instrument (Shanghai, China).

HPLC-grade methanol was obtained from the Shanghai Chemical Reagent Company (Shanghai, China). Purified water was obtained with a Milli-Q apparatus (Millipore, Bedford, MA).

2.2. Instrumentation. All GC/MS analysis was performed using a Shimadzu GCMS-QP2010 plus (Kyoto, Japan) equipped with an AOC-20i auto injector (Kyoto, Japan). The software for data acquisition and processing was GCMSsolution version 2.6. Design of the monolithic material sorptive extraction-MonoTrap (RCC18) used for sample preparation is based on monolithic technology (Merck KGaA, Darmstadt, Germany). It is a new hybrid adsorptive material with a large surface area of 150 m^2/g or more, due to its monolithic structure: activated carbon present inside and outside the frame and octadecylsilyl (ODS) groups chemically bonded to its surface. These factors work in unison to provide a high level of efficiency.

2.3. Sample Preparation. Rice florets were collected from fresh rice plants and stored at −20 °C. Other flowers (jasmine, wild chrysanthemum, osmanthus, and rose) were purchased from a local supermarket, and stored at room temperature until analysis. The flower samples were crushed when they were used. Samples (1.0 g) of the powder were further homogenized in methanol (25 mL). The admixture was subjected to ultrasound homogenization for 1 h, and then methylated by the addition of 100 μL of (trimethylsilyl)-diazomethane and incubated at room temperature for 5 min. The methylated sample was evaporated to dryness under a stream of N_2 before further extraction.

2.4. Pretreatment Involving MMSE. The procedure is shown in Supporting Information Figure S1. It involved placing the MT Holder on the MT Stand. The MonoTrap was picked up using tweezers and the holder was inserted into the hole on the MonoTrap. The MT Holder was then held using pliers whose ends had been cleaned, and the MT Holder was then passed through the septum on the vial. A cap was placed on the top of the MT Holder, and the septum on the vial was then tightened. The vial was placed in an oven heated to 130 °C and incubated at that temperature for 60 min. After the incubation was complete, the MT Extract Cup was filled with the extraction solvent (200 μL of ethyl acetate), the septum was tightened, the vial was placed in an ultrasonic cleaner and exposed to ultrasound for 10 min to accelerate the extraction, and the solution was then injected directly into the GC/MS instrument, using an injection volume of 1 μL.

2.5. GC/MS Analysis. The separation was achieved using a chiral capillary column (Rt-bDEXsm, 30 m × 0.25 mm i.d.; film thickness 0.25 μm) (Restek International, USA). The oven temperature was held at 80 °C for 1 min at the start of run, then increased to 160 °C at a rate of 8 °C/min, and then increased to 180 °C at 3 °C/min, before being held at 180 °C for 8 min. The split injection mode was used, and the split ratio was 20:1. Helium (purity 99.999%) with a flow rate of 1 mL/min was used as carrier gas. The injection port, ion source, and interface temperatures were all held at 200 °C. The electron ionization (EI) mass spectra of the analytes were recorded by scan mode (scan range: m/z 50−500) to determine retention times and characteristic fragment ions. For quantitative analysis, the chosen characteristic fragment ions were monitored in the selected-ion monitoring (SIM) mode, with characteristic ions of m/z 83, 151, and 224 for methyl jasmonate. Ion ratio acceptance criterion was a deviation ≤30% ion ratio from the calibration sample.

3. RESULTS AND DISCUSSION

3.1. Separation of Methyl Jasmonate Enantiomers Following MMSE Pretreatment. Our approach enabled baseline separation of the four enantiomers of methyl jasmonate. As shown in Figure 2, a methyl jasmonate chromatogram was obtained by MMSE−GC/MS from a sample of standard solution that had been methylated (1000 ng/mL). The peak sequence was identified according to a previous study,[28] with the four enantiomers (−)-(3*S*,7*R*)-methyl epi-jasmonate, (−)-(3*R*,7*R*)-methyl jasmonate, (+)-(3*S*,7*S*)-methyl jasmonate, and (+)-(3*R*,7*S*)-methyl epi-jasmonate. A baseline separation for four enantiomers of methyl jasmonates had been achieved with retention times at 21.29, 21.87, 22.53, and 23.45 min, respectively. The respective resolution factors of the four enantiomers were 2.09, 1.58, and 1.55. The separation of methyl jasmonate enantiomers following MMSE pretreatment seems to be easier and more convenient than the existing method.[28]

3.2. Optimization of Conditions for MMSE. Conditions such as the extraction temperature, extraction time, extraction solvent, and duration of exposure to ultrasound needed to be optimized in order to ensure the best sorptive extraction efficiency of the MonoTrap for methyl jasmonate.

The extraction temperature was monitored by increasing the temperature of the oven from 90 to 160 °C. It can be seen in

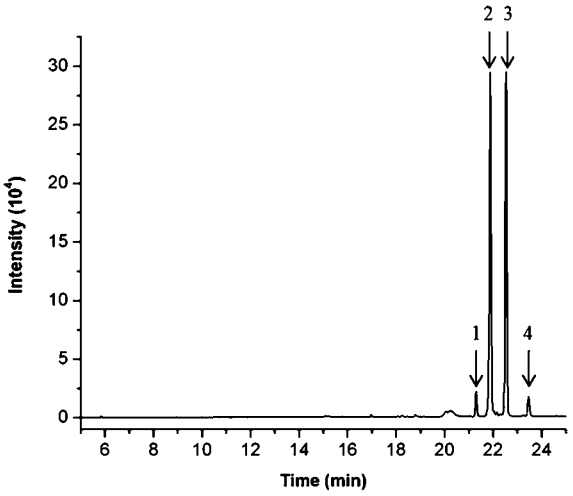

Figure 2. Chromatogram resulting from the MMSE–GC/MS of methyl jasmonate (1000 ng/mL): **1**, (−)-(3*S*,7*R*)-methyl epi-jasmonate; **2**, (−)-(3*R*,7*R*)-methyl jasmonate; **3**, (+)-(3*S*,7*S*)-methyl jasmonate; **4**, (+)-(3*R*,7*S*) methyl epi-jasmonate. Conditions are described under Materials and Methods.

Figure 3A that the amount of methyl jasmonate enantiomers extracted increased as the temperature of the oven increased, and then remained stable. The extraction efficiency did not increase at temperatures higher than 130 °C. This can be explained by boiling point of methyl jasmonate (110 °C). At 130 °C, almost all the methyl jasmonate can be extracted, so a temperature of 130 °C was chosen for all subsequent extraction procedures.

The extraction time was optimized in the range of 15–90 min. As shown in Figure 3B, the extraction efficiency increased with time until after more than 60 min, when no further extraction was evident. This can be explained by each MonoTrap having a limited capacity for extraction, and being unable to absorb more methyl jasmonate after exceeding its extraction capacity. Given that the reaction reached its maximum extraction capacity at 60 min, this duration was selected as the optimal time for the extraction process.

The extraction solvent was selected by comparison of the effectiveness of several solvents. Among these solvents, ethyl acetate provided the most efficient extraction (Figure 3C). The best duration for exposure to ultrasound was 10 min, after which time the solution could be used directly for GC/MS analysis.

3.3. Selection of Quantitative Ions and Qualitative Ions. The application of the GC/MS method to determine methyl jasmonate levels was verified using an external standard method for quantitation. First, the analytical performance of the optimized GC/MS was determined using a standard solution that had been methylated. After the scan mode was applied to determine retention times and characteristic fragment ions, the SIM mode was employed to achieve suitable sensitivity. The mass spectra of four enantiomers of methyl jasmonate are shown in Figure 4. A fragment of *m*/*z* 83 was selected as the quantification ion of the target because of its high sensitivity and the fact that there was no interference peak in this region at the retention time near methyl jasmonate. Reference ions of *m*/*z* 151 and 224 were also monitored. The ion ratios used (*m*/*z* 83/151/224) for confirmatory purpose was studied in all sample matrices. Similarity index (SI) between raw spectra data

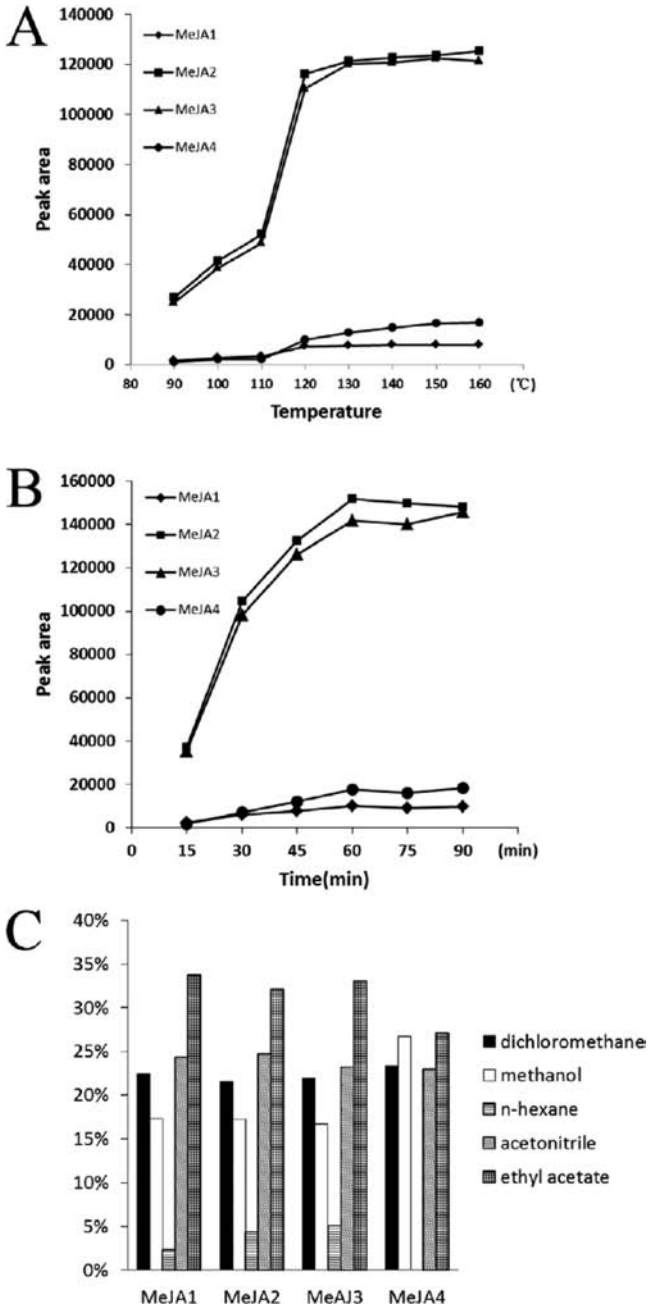

Figure 3. (A) Effect of extraction temperature on peak areas of methyl jasmonate. (B) Influence of extraction time on MMSE. (C) Evaluation of different solvents on methyl jasmonate recoveries by MMSE.

and those in NIST08 library calculated by the software were exceeding 90%.

3.4. Method Validation. The application of the MMSE–GC/MS method for the determination of methyl jasmonate was verified using an external standard for quantification. The external calibration was performed by adding different standard samples. The sample solutions were spiked with stock solution to get final concentrations of 1, 2, 5, 10, 20, 50, and 100 ng/mL. The SIM mode was employed to achieve suitable sensitivity. The calibration curve used to ensure the requisite level of sensitivity was described by the equation $Y = 2\,969\,892X + 1556.782$ (where Y = peak area, X = concentration of compounds) with a correlation coefficient (R) >0.999. Detection and quantification limits were calculated as the

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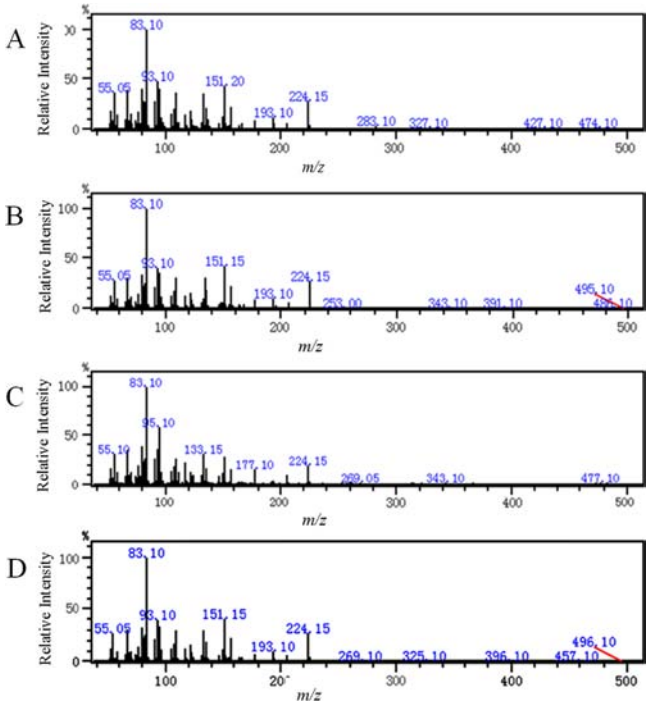

Figure 4. Mass spectra of four enantiomers of methyl jasmonate: (A) (−)-(3*S*,7*R*)-methyl epi-jasmonate; (B) (−)-(3*R*,7*R*)-methyl jasmonate; (C) (+)-(3*S*,7*S*)-methyl jasmonate; (D) (+)-(3*R*,7*S*)-methyl epi-jasmonate. Experimental conditions are the same as those for Figure 2.

concentration corresponding to a signal 3 and 10 times the standard deviation of the baseline noise, respectively. The limit of detection was 0.257 ng/mL. The limit of quantification was 0.856 ng/mL.

The extraction recoveries were determined by analysis of the spiked methyl jasmonate standard solution at different concentrations. Samples were spiked with 2 and 50 ng/mL methyl jasmonate. The recoveries were calculated by comparing the extracted amounts of methyl jasmonate from those of the samples, with the corresponding spiking amounts determined using calibration curves. The recoveries spiked with 2 and 50 ng/mL were 116.8% and 99.4%, and relative standard deviations (RSDs) were 13.7% and 1.1%, respectively.

The reproducibility of the developed method was determined by the inter- and intraday precision. The precision was acquired by samples spiked at three levels of concentration (2, 10, 50 ng/mL). Five extractions of a sample solution over a day gave the intraday RSDs; while interday precisions data were obtained by analysis of the samples extracted on five consecutive days. Excellent method reproducibility was achieved. The intraday precisions for 2, 10, 50 ng/mL were found to be 6.9, 6.2, and 5.2%, respectively; the interday precisions for 2, 10, 50 ng/mL were 9.7, 8.9 and 8.8%, respectively.

3.5. Quantitative Analysis of Jasmonic Acid and Its Methyl Ester in Flowers. Based on the procedures described above, the MMSE−GC/MS technique was applied for the analysis of jasmonic acid and its methyl ester in flowers. Five flower samples were analyzed, and the contents of jasmonic acid and methyl jasmonate in these samples are shown in Table 1. The contents of methyl jasmonate were calculated by the direct determination without derivatization. The contents of jasmonic acid were calculated by subtracting the content after derivatization with the data without derivatization. The typical chromatogram for analysis of rice-flower is shown in Figure 5. It

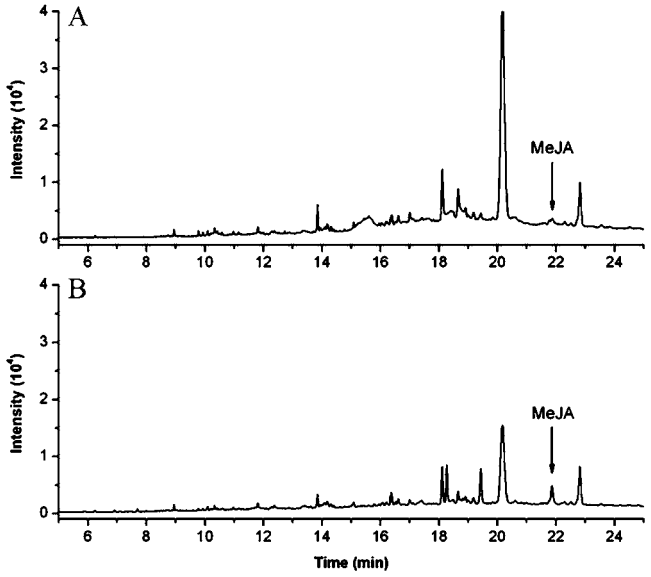

Figure 5. Typical chromatogram for analysis of jasmonates by GC/MS without (A) and with methylated derivatization (B). Experimental conditions are the same as those for Figure 2.

is obvious that only one enantiomer can be detected in the real sample. The recovery for five flower samples spiked with 2 ng/mL and 50 ng/mL was examined, and all samples obtained were analyzed with three replicates. As shown in Table 1, RSDs were less than or equal to 15.0%.

Table 1. Content, Recoveries, and Relative Standard Deviation (RSDs) of Methyl Jasmonate in Five Flower Samples

	content (ng/mL)					
sample	jasmonic acid	methyl jasmonate	total	spiked amount (ng/mL)	recovery (%)	RSD (%)
rice flower	8.96	1.09	10.05	2	87.2	13.8
				50	72.2	15.0
jasmine flower	1.22	1.07	2.19	2	70.4	14.6
				50	62.0	6.3
wild chrysanthemum	9.28	5.30	14.58	2	67	7.8
				50	65.2	14.9
osmanthus	2.82	5.01	7.83	2	75.1	14.9
				50	67.7	13.4
rose	1.83	1.28	3.11	2	77.1	10.4
				50	66.0	10.3

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The combination of MMSE using a MonoTrap with GC/MS provides a simple, sensitive, and selective procedure for the identification and determination of methyl jasmonate in flowers. The MonoTrap enables excellent extraction of methyl jasmonate. The MMSE method described provides an appropriate and rapid sample pretreatment technique with the potential to be applied to determine the levels of other plant hormones.

■ ASSOCIATED CONTENT

Supporting Information

Figure S1: Pretreatment procedure involving MMSE. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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