JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

UHPLC–MS Analysis of Juvenile Hormone II in Mediterranean Corn Borer (*Sesamia nonagrioides*) Hemolymph Using Various Ionization Techniques

Francisca Vilaró,[†] Meritxell Pérez-Hedo,[‡] Jordi Eras,*[,†,§] Ramon Canela,[§] and Matilde Eizaguirre[‡]

[†]Scientific Technical Services, University of Lleida, PCiTAL, 25003-Lleida, Spain

[‡]R+D Center (UdL-IRTA), Rovira Roure 191, 25198 Lleida, Spain

[§]Chemistry Department, ETSEA, University of Lleida, 25198-Lleida, Spain

Supporting Information

ABSTRACT: The juvenile hormones (JHs) have been considered the most versatile hormones in the animal kingdom. JH-II is the most abundant JH in *Sesamia nonagrioides*, important maize pests in the Mediterranean basin. This study compared the sensitivities and matrix effects of four ionization modes on analyzing JH-II in *S. nonagrioides* hemolymph using ultrahigh-performance liquid chromatography–mass spectrometry (UHPLC–MS) in single ion monitoring (SIM) mode. The ionization techniques tested were electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI), and APPI with the lamp turned off, which corresponds to atmospheric pressure thermospray ionization (APTSI). ESI was discarded because of the high matrix effect. APPI was discarded because the correlation responses between solvent and matrix on the instrumental quality parameters were worse than those for APTSI and APCI. In our analytical conditions, APCI has shown the best validation parameter values. APCI ionization is widely available in instrumental laboratories.

KEYWORDS: *juvenile hormone (JH), ultra high-performance liquid chromatography (UHPLC), electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI), atmospheric pressure thermospray ionization (APTSI)*

INTRODUCTION

The Mediterranean corn borer, *Sesamia nonagrioides* (Lef.) (Lepidoptera: Noctuidae), is one of the two most damaging corn borers in the Mediterranean basin. This noctuid feeds on maize, sugar cane, and millet in Southern Europe and Northern Africa. The number of generations per year that the species has in a region depends on the time when diapause induction conditions, mainly short days and low temperature, occur. *S. nonagrioides* has 2–4 generations in its spreading area.[1−3] Its larvae display an unusual type of diapause, which shares some characteristics with the diapause of other cereal borers. While a decline of juvenile hormone (JH) levels in developed nondiapausing larvae allows the pupation and the occurrence of the next adult generation, diapausing larvae maintain a high level of JH in the hemolymph that prevents pupation. Moreover, the high level of JH allows diapausing larvae to molt periodically but still maintain the larval stage.[4,5] Besides the environmental factors that induce diapause, other factors can modify the level of JH in the larvae such as the ingestion of toxins with the plant.[6] Thus, the determination of the level of JH in the larvae is important to understand the larval development of this species and others with similar diapause features.

JH-II is the most abundant JH in this species.[4,5] This compound, as for other JHs, is an acyclic sesquiterpenoid that regulates important processes related to the development, production of eggs in the female's ovaries, diapauses, and other aspects of the physiology of the insects. The determination of the JH levels in different developmental stages is essential to study the different aspects of insect development, to obtain new bioanalogue derivatives of the different JHs,[7] and to apply this knowledge to pest control.[8]

Numerous analytical techniques have been used to detect and quantitate JH in hemolymph. Although the most commonly used techniques are based on gas chromatography–mass spectrometry (GC–MS), they involve several steps of intense purification and a derivatization step prior to analysis.[9,10] Radioimmunoassay is another analytical technique used for quantitative analysis of JHs,[8,11−13] but these methods can give a false estimation of the JH level because some lipids may interfere with results even after the purification steps[14] that are necessary for quantification of hemolymph samples.[15]

In recent years, liquid chromatography–mass spectrometry (LC–MS) with electrospray ionization (ESI) in selected ion monitoring (SIM) mode, recording the sodium adduct $[M+Na]^+$ because of the high abundance of Na^+ in insect hemolymph, has become a powerful analytical technique that permits the determination of JHs and some of their metabolites.[16] Moreover, this technique offers at least the same accuracy and precision as GC–MS despite the fewer purification steps that are needed. Consequently, LC–MS is a less time-consuming technique when compared to GC–MS.[14,16]

Received: November 11, 2011
Revised: February 28, 2012
Accepted: March 1, 2012
Published: March 1, 2012

Two other ionization techniques are commercially available in LC−MS, atmospheric pressure chemical ionization (APCI) and, more recently, atmospheric pressure photoionization (APPI), which has been applied in recent years to a broad range of compounds including lipids,[17] polycyclic aromatic hydrocarbons,[18] and carbamates,[19] among others. The APPI source provides, when its lamp is turned off, atmospheric pressure thermospray ionization (APTSI) conditions.

This study was undertaken to investigate four ionization methods: ESI, APCI, APPI, and APTSI for monitoring JH-II in hemolymph extracts of *S. nonagrioides* larvae. The sodium adduct ion was recorded in positive mode in all ionization techniques because of its abundance in hemolymph samples. We evaluated and compared signal intensity and matrix effects in these ionization methods for JH-II analysis.

MATERIALS AND METHODS

Hemolymph Sample Extraction. JH-II extraction was performed according to the Westerlund and Hoffmann method[16] with slight modifications. Twenty microliters of sixth instar *S. nonagrioides* larvae hemolymph was collected with a glass capillary and placed in methanol/isooctane (1:1, v/v) with 1.8 ng of methoprene as internal standard. The hemolymph/solvent ratio was 1:50 (v/v). This mixture was mixed quickly for 20 s and allowed to stand at room temperature for 30 min. Next, each sample was centrifuged at 8500*g* for 15 min, and the isooctane phase was transferred to a glass vial. The methanol phase was mixed again, centrifuged at 10 000*g* for 30 min, and combined with the isooctane phase in the same vial. The extracts were stored at −80 °C or concentrated under nitrogen flow and diluted with 100 μL of methanol/water (80:20, v/v) for immediate analysis. The extracts were injected in duplicate.

Preparation of Standard. Stock solutions of JH-II and methoprene were separately prepared in methanol. All standard solutions were stored at −80 °C. Each stock solution was then diluted with methanol/water (80:20, v/v) to prepare a set of standard solutions. A five-point calibration curve was obtained by spiking sample blank extract in methanol/water (80:20, v/v), free from JH-II to cover a 0.5−50 ng/mL range with 18 ng/mL of methoprene as internal standard (IS). Each experimental value corresponds to the average of three independent measurements.

Chemicals. JH-II (methyl-(±)-(2*E*,6*E*)-10,11-epoxy-3,7,11-trimethyl-2,6-tridecadienoate) 78% by synthetic origin was purchased from SciTech (Prague, Czech Republic). Methoprene (technical material) (isopropyl-(2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) 8.0% *cis* 90.1% *trans* was purchased from Sigma-Aldrich Quimica S.A. (Madrid, Spain). Figure 1 shows the structures of JH-II and methoprene. All solvents were of Prolabo LC−MS grade (Llinars del Vallès, Spain). Water was prepared using a Millipore Synergy UV system (Madrid, Spain).

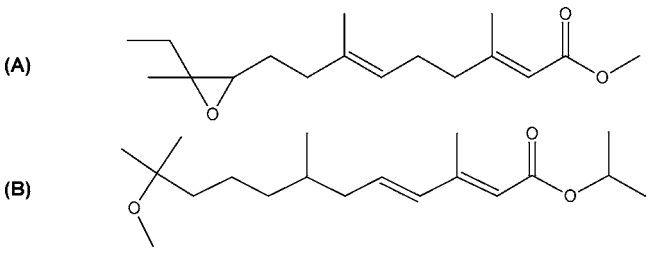

Figure 1. Chemical structures of (A) JH-II with M_W 280.4 and (B) methoprene with M_W 310.5.

Instrumental Parameters. The ultra high-performance liquid chromatography−mass spectrometry (UHPLC−MS) system utilized was a Waters Acquity UPLC (Waters, Milford, MA) coupled with a Waters Acquity TQD triple quadrupole mass spectrometer (Waters, Manchester, UK) using ESI, APCI, and APPI interfaces. The system was operated under MassLynx 4.1 software (Waters Corp., Milford, MA).

The chromatographic separations were carried out at 28 °C in isocratic mode using methanol/water (80:20, v/v) as the mobile phase. The injection volume was 15 μL in a partial loop with needle overfill. The column used was a 100 mm × 2.1 mm i.d., 1.7 μm, Acquity UPLC BEH C18 (Waters) at a flow rate of 400 μL/min. A total separation time of 7 min was needed.

The effluent from the UHPLC separation was introduced in-line into a mass detector operating in positive ion mode. The MS optimized operating parameters for each interface source are listed in Table 1.

Quantification was performed by the IS method with monitoring in the SIM mode $[M+Na]^+$ for JH-II and methoprene (IS).

Matrix Effects and Validation Procedures. To compare the matrix effect using different ionization sources, a standard-addition approach was used with blank sample extracts.[20,21] Blank samples, lacking JH, were obtained according to Pérez-Hedo et al.[22] Thirty-six decapitated sixth instar *S. nonagrioides* larvae deprived of their *corpora allata* were maintained alive 4 days after the surgical procedure. The JH has disappeared from the hemolymph after this period.[23] Consequentely, 720 μL of hemolymph obtained from these insects was subjected to the method described above. The matrix effect was evaluated by comparing the variation of responses of the analytes in neat methanol/water (80:20, v/v) (A) and in standards spiked into blank sample extracts (B). The matrix effect values were calculated as follows:

$$\text{matrix effect} = B/A \times 100 \quad (1)$$

To validate the analytical method, we determined the solvent and matrix-matched instrumental quality parameters, such as linearity, calibration curve, limit of detection (LOD), limit of quantification (LOQ), and instrumental accuracy (precision and accuracy).

RESULTS AND DISCUSSION

The aim of this study was to evaluate the potential of different ionization techniques in UHPLC−MS for the identification and quantification of JH-II in insect hemolymph extracts. To evaluate which source was more suitable for this analysis, the performances of ESI, APCI, APPI, and APTSI were compared. In all cases, we recorded $[M+Na]^+$ in SIM mode because it was not possible to break down this adduct as a result of its high stability. This permits the adaptation of the method to single quadrupole mass spectrometers.

Chromatographic Method. Figure 2 shows representative chromatograms of a standard, a matrix blank spiked with JH-II and methoprene, and a matrix blank. This figure shows that the matrix interferences were well resolved for the target compound and no interferences were present for the IS. In our case, the total analysis time for chromatographic separation was 7 min, which is much shorter than the chromatographic method developed for JH-II without methoprene,[16] which needed 20 min for carrying out the analysis, and the method developed for JH-III with methoprene (35 min).[24]

Optimization of MS Operating Parameters. To obtain the maximum intensity value for JH-II, we determined the optimum values of the operating parameters for each source. These studies were carried out combining a direct infusion of JH-II (1 μg/mL in methanol/water, 80:20 v/v at 5 μL/min) with the mobile phase at the working flow rate. Table 1 shows the optimum values for the operating parameters and the ranges tested for each ionization source. Ionization mode selection and method validation were determined with the optimum values of the main parameter settings.

Table 1. Optimum Values of the Main Parameter Settings for Different Ionization Sources[a]

conditions	ESI	APCI	APPI/APTSI
capillary (kV)	3.5 (1.5−4)		
corona (kV)		0.6 (0.1−1.0)	
repeller (kV)			1.5 (0.1−2.0)
lamp			on/off
cone (V)	20 (5−40)	20 (5−40)	20 (5−40)
extractor (V)	3	3	3
RF lens (V)	0.1	0.1	0.1
source temperature (°C)	120 (80−150)	120 (80−150)	120 (80−150)
probe temperature (°C)		400 (200−500)	400 (200−500)
desolvation temperature (°C)	400 (200−500)		
cone gas flow (L/h)	10 (0−20)	5 (0−20)	5 (0−20)
desolvation gas flow (L/h)	700 (400−900)	150 (100−200)	150 (100−200)

[a]The parameter ranges studied are shown in brackets.

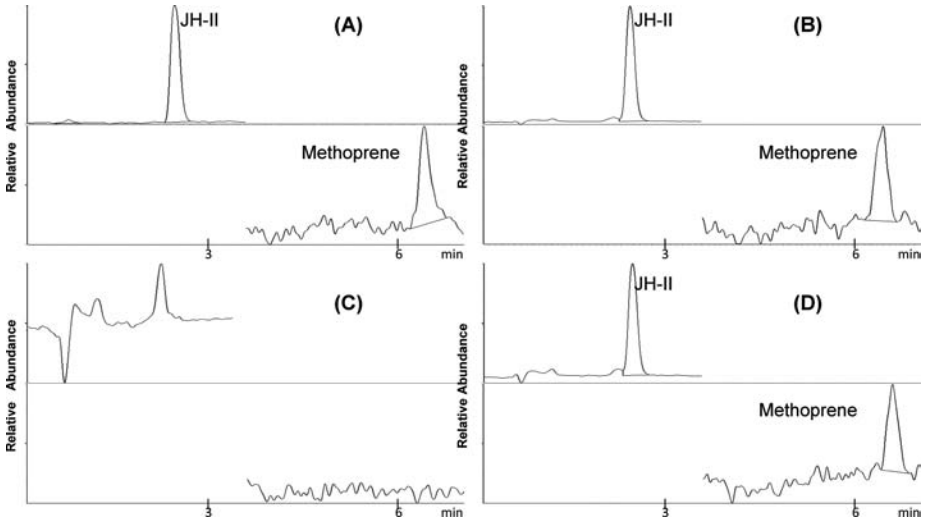

Figure 2. Ion chromatograms of (A) JH-II and methoprene in methanol/water (80:20, v/v), (B) matrix blank spiked with JH-II and methoprene, (C) matrix blank, and (D) real sample.

Ionization Mode Selection. Previous studies[14,16,24] have been performed by ESI ionization. We first examined the spectra of JH-II and methoprene in methanol/water (80:20, v/v) and in matrix-matched samples using different ionization modes (Figure 3). These spectra illustrate the ion fragmentation in different conditions and demonstrated that in the ESI mode the spectra in solvent and in samples were very different.

Recently, Miyazaki et al.[24] analyzed JH-III in whole body extracts of Formosan termite workers by liquid chromatography−ion trap mass spectrometry using an ESI source. They overcame the differences in solvent and matrix spectra by employing a gradient of acetonitrile/water as a mobile phase with 1 mM ammonium acetate as a supplement to enhance ionization of JH-III.

Table 2 shows the results obtained comparing the response of JH-II (area JH-II/area methoprene) using ESI, APCI, APPI without doping agent, and APTSI (APPI lamp off) in solvent and matrix. Four concentrations of JH-II were analyzed recording at SIM mode m/z 303 for JH-II and m/z 333 for methoprene. With all four ionization techniques, both JH-II and methoprene were invariably detected as the sodium adduct $[M+Na]^+$. However, the relative intensities of the two compounds were found to be highly dependent on the specific ionization technique employed. In addition, a very important matrix effect was found in ESI ionization. This matrix effect produces an overestimation of JH-II contents in samples. On the other hand, APPI presents a slope and intercept higher than the other two ionization techniques and a correlation coefficient slightly lower. APCI and APTSI ionization techniques show correlations greater than 0.99, slopes of around 1, and lower intercepts.

In an attempt to enhance APPI signals, we tested the addition of a dopant (acetone, toluene, and anisole). However, a strong decrease of signal/noise value in all experiments was observed (data not shown). Finally, we carried out the determination of the analytical quality parameters to select the best ionization technique for this quantitative analysis.

Evaluation of Matrix Effects. The matrix can be one of the main factors that cause a decrease in method sensitivity and accuracy and if it is not well characterized may lead to erroneous quantification results.

The matrix effects were evaluated using the postextraction standard-addition method, which is based on the comparison of the response of JH-II in solvent and when matrix matched (n = 4). In this context, a slope value >1 indicates ionization enhancement, whereas a slope value <1 indicates ionization suppression (Table 2). With the use of this approach, hemolymph extracts were spiked with three different amounts of JH-II and analyzed by UPLC−APCI-MS and UPLC−APTSI-MS in SIM mode. The analyses were repeated on four

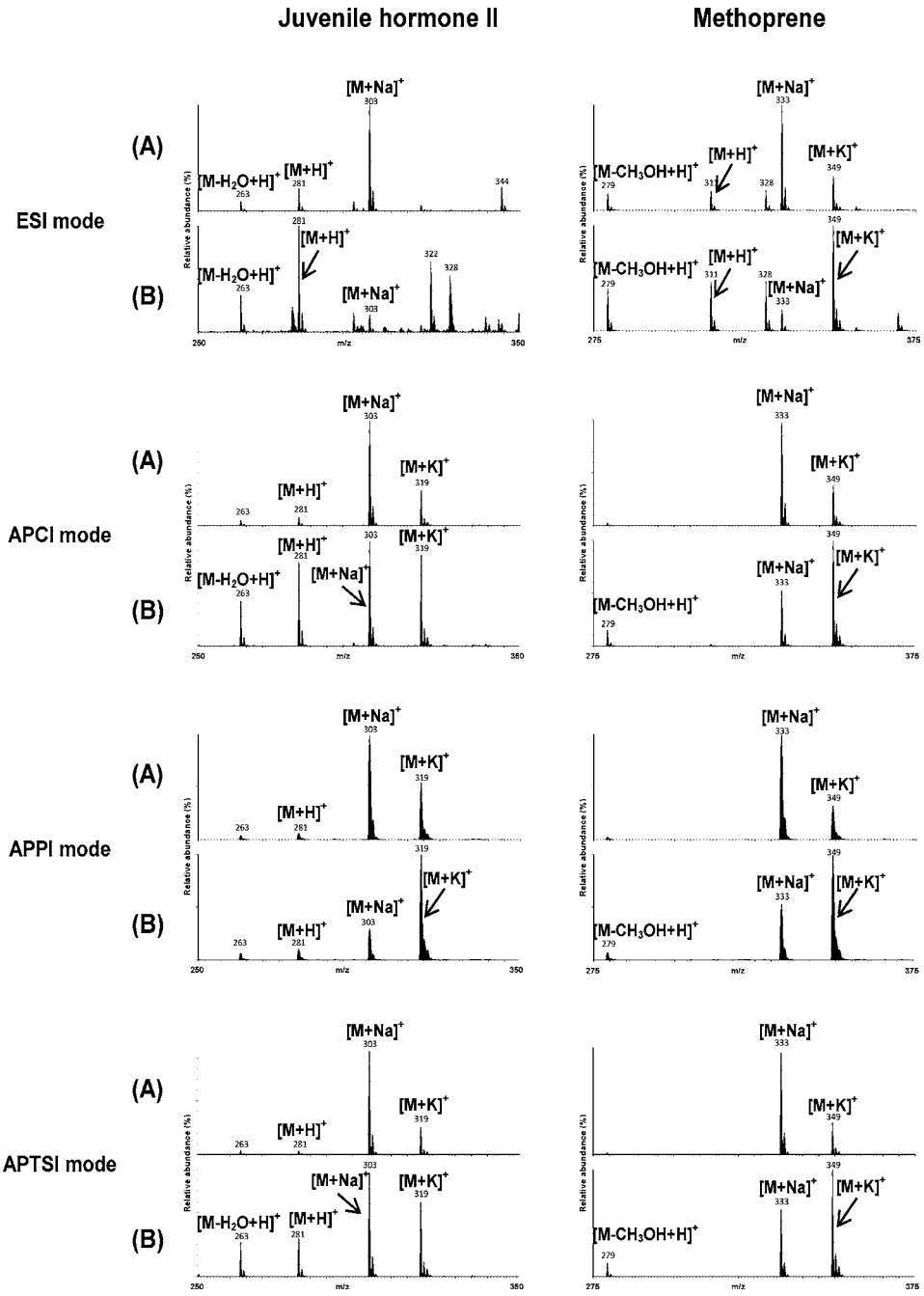

Figure 3. MS spectra of JH-II and methoprene obtained using different ionization modes (A) in blank matrix and (B) in solvent.

replicates to assess the matrix effects. The matrix effects of these ionization techniques were slightly different, as shown in Table 3. JH-II suffered a suppression of between 11% and 19%

Table 2. Correlation of Relative Responses (JH-II/Methoprene) in Solvent and Matrix Matched Using Different ionization Techniques

	correlation parameters		
ionization technique	slope	intercept	R^2
APCI	0.832	0.142	0.999
APTSI	0.525	−0.365	0.995
APPI	0.485	−0.609	0.990
ESI	18.966	−13.297	0.899

Table 3. Matrix Effects Evaluation (Average ± RSD%) in *S. nonagrioides* Hemolymph Using APCI and APTSI Ionization Techniques

level	spiked JH-II (ng/mL)	APCI ionization	APTSI ionization
low	0.84	89 ± 8	81 ± 6
medium	3.35	89 ± 7	85 ± 9
high	11.18	94 ± 5	89 ± 7

in APTSI and between 6% and 11% in APCI in hemolymph extracts. The experimental data show that APCI ionization allows the analysis of JH-II regardless of the presence of coeluting interferences.

Calibration Integrity, Lower Limits. Calibration curves were obtained by least-squares linear-regression analysis of the

dx.doi.org/10.1021/jf204621h | J. Agric. Food Chem. 2012, 60, 3020−3025

peak area ratio of JH-II to methoprene (IS) versus JH-II concentration (Table 4). Six standard solutions were employed

Table 4. Linear Regression Data, Limits of Detection (LOD), and Quantification (LOQ) Using APCI and APTSI Ionization Techniques

	APCI ionization		APTSI ionization	
parameter	matrix matched	solvent	matrix matched	solvent
linear range (ng/mL)	0.5−50	0.5−50	0.5−50	0.5−50
slope ± SD	3.11 ± 0.27	3.45 ± 0.47	3.39 ± 0.67	3.57 ± 0.61
intercept ± SD	0.06 ± 0.19	0.41 ± 0.26	0.05 ± 0.46	0.45 ± 0.47
R^2	0.994	0.994	0.996	0.996
LOD (ng/mL)	0.20	0.25	0.45	0.43
LOQ (ng/mL)	0.61	0.75	1.36	1.32

to determine the calibration curves, and two replicate measurements were carried out for each standard solution. Linearity was derived from calibration curves (n = 4). For both techniques (APCI and APTSI), the calibrations were linear over the entire work range (0.5−50 ng/mL, $R^2 \geq 0.994$). The standard deviations (SDs) of the slopes and intercepts show that APCI is more stable than APTSI.

Sensitivity was evaluated by determining the LODs and LOQs. Both parameters have been calculated using the equations LOD = $3.3 \cdot S_b/a$ and LOQ = $10 \cdot S_b/a$, where a is the slope and S_b is the SD of the intercept.[25] These results show that APCI employing matrix matching as the calibration standard was the most sensitive ionization technique. Values of 0.20 and 0.61 ng JH-II/mL hemolymph extract were calculated for LOD and LOQ, respectively.

Instrumental Accuracy. Table 5 shows the instrumental precision and accuracy. We determined these parameters as a measurement repeatability (n = 6) of five levels with various concentrations. The precision was specified as relative standard deviation (RSD% = SD × 100/average). Although both ionization techniques show acceptable precision and accuracy values, the more accurate ionization technique was APCI using matrix matching as the calibration standard. RSD% values should not exceed 15% (20% near LOQ). On the other hand, the accuracy was expressed as relative error (Er% = [experimental value − true value] × 100/true value). For acceptance, Er% values should range between −50% and +20% when the target compound content is equal to or lower than 1 μg/kg, which is our case.[26]

In conclusion, this work shows that UHPLC−APCI-MS could be a fast and valuable tool for quantitative determination of JH-II in *S. nonagrioides* hemolymph extracts. The analysis was carried out in positive ionization mode under SIM recording. Although previous LC approaches permitted the determination of JHs in hemolymph extracts, the new UHPLC method allowed a significant reduction in analysis time, implying also a reduction in solvent consumption. The instrumental throughput is significantly increased, with a run in 7 min. In consequence, the previous HPLC[15,23] method involving several gradient steps can be substituted by a UHPLC isocratic method. We exhaustively investigated the performance of ESI, APPI, APCI, and APTSI. We observed a huge advantage for an APCI source over an ESI source because of the high matrix influence in ESI spectra. The APPI response correlations between solvent and the spiked matrix (four levels) display worse correlation parameters (slope, intercept, and R^2) than the ones obtained using the APCI and APTSI ionization techniques. Consequently, the APCI and APTSI ionization modes show a better performance. Moreover, our work shows that APCI is superior to APTSI in terms of matrix effect, linearity, LOD, LOQ, and instrumental accuracy. Furthermore, the use of an APCI source presents an additional advantage; in contrast to the APPI source, the APCI source is a common technique in most instrumental laboratories having an LC−MS system. Finally, if we compared the values of LOD, LOQ, instrumental precision, and accuracy validation parameters, we can conclude that APCI with matrix-matched calibration curve is the best method for quantitative determination of JH-II in *S. nonagrioides* hemolymph.

Finally, the developed method has been successfully used to measure small variations in the JH levels in the larvae of *S. nonagrioides* because of Bt maize ingestion.[6]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Correlation of relative responses (JH-II/methoprene) in solvent and matrix matched using different ionization techniques (each point corresponds to the average of three independent values). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: +34 973 702589. Fax: +34 973 238264. E-mail: eras@quimica.udl.cat.

Table 5. Instrumental Precision and Accuracy for APCI and APTSI Ionization Techniques Using Matrix Matched Calibration Curve and Solvent Calibration Curve

	APCI ionization				APTSI ionization			
	matrix matched		solvent		matrix matched		solvent	
level (ng/mL)	precision (RSD%)	accuracy (Er%)	precision (RSD%)	accuracy (Er%)	precision (RSD%)	accuracy (Er%)	precision (RSD%)	accuracy (Er%)
1.1	7.5	−4.0	17.8	−6.0	ND[a]	ND	ND	ND
2.2	6.4	2.3	16.3	−3.9	10.1	7.1	13.7	−7.1
4.5	2.0	−6.6	7.7	−1.2	6.5	6.8	17.7	5.2
9.0	4.5	0.6	4.4	−0.3	3.8	−5.3	5.2	2.6
13.5	5.5	4.5	3.4	1.2	2.4	0.3	10.8	−7.3

[a]Not determined. Level lower than LOQ.

Funding

This work has been partially funded by the Spanish R&D Agency (CICYT) through the project AGL2008-02355.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to the Scientific Technical Services for providing analytical instruments for this study.

■ ABBREVIATIONS USED

APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photoionization; APTSI, atmospheric pressure thermospray ionization; Er%, relative error; ESI, electrospray ionization; GC−MS, gas chromatography−mass spectrometry; IS, internal standard; JH, juvenile hormone; LC−MS, liquid chromatography−mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; RSD%, relative standard deviation; SD, standard deviation; UHPLC−MS, ultra high-performance liquid chromatography−mass spectrometry

■ REFERENCES

(1) Eizaguirre, M.; Albajes, R. Diapause induction in the stem corn borer, *Sesamia nonagrioides* (Lepidoptera: Noctuidae). *Entomol. Gen.* **1992**, *17*, 277−283.

(2) Eizaguirre, M.; López, C.; Sans, A. Maize phenology influences field diapause induction of *Sesamia nonagrioides* (Lepidoptera Noctuidae). *Bull. Entomol. Res.* **2002**, *92*, 439−443.

(3) Fantinou, A. A.; Karandinos, M. G.; Tsisipis, J. A. Diapause induction in the *Sesamia nonagrioides* (Lepidoptera: Noctuidae) effect of photoperiod and temperature. *Environ. Entomol.* **1995**, *24*, 1458−1466.

(4) Eizaguirre, M.; Prats, J.; Abellana, M.; López, C.; Llovera, M.; Canela, R. Juvenile hormone and diapause in *Sesamia nonagrioides*. *J. Insect Physiol.* **1998**, *44*, 419−425.

(5) Eizaguirre, M.; Schafellner, C.; López, C.; Sehnal, F. Relationship between an increase of juvenile hormone titer in early instars and the induction of diapause in fully grown larvae of *Sesamia nonagrioides*. *J. Insect Physiol.* **2005**, *51*, 1127−1134.

(6) Pérez-Hedo, M.; Albajes, R.; Eizaguirre, M. Modification of hormonal balance in larvae of the corn borer *Sesamia nonagrioides* (Lepidoptera: Noctuidae) due to sublethal *Bacillus thuringiensis* protein ingestion. *J. Econ. Entomol.* **2011**, *104*, 853−861.

(7) Wimmer, Z.; Jurcek, O.; Jedlicka, P.; Hanus, R.; Kuldova, J.; Hrdy, I.; Bennettova, B.; Saman, D. Insect pest management agents: Hormonogen esters (juvenogens). *J. Agric. Food Chem.* **2007**, *55*, 7387−7393.

(8) Kai, Z.; Xie, Y.; Huang, J.; Tobe, S. S.; Zhang, J.; Ling, L.; Zhang, L.; Zhao, Y.; Yang, X. Peptidomimetics in the discovery of new insect growth regulators: Studies on the structure-activity relationships of the core pentapeptide region of allatostatins. *J. Agric. Food Chem.* **2011**, *59*, 2478−2485.

(9) Bergot, B. J.; Ratcliff, M.; Schooley, D. A. Method for quantitative determination of the four known juvenile hormones in insect tissue using gas chromatography-mass spectroscopy. *J. Chromatogr.* **1981**, *204*, 231−244.

(10) Rembold, H.; Lackner, B. Convenient method for the determination of picomole amounts of juvenile hormone. *J. Chromatogr.* **1985**, *323*, 355−361.

(11) Goodman, W. G.; Orth, A. P.; Toong, Y. C.; Ebersohl, R.; Hiruma, K.; Granger, N. A. Recent advances in radioimmunoassay technology for the juvenile hormones. *Arch. Insect Biochem. Physiol.* **1995**, *30*, 295−306.

(12) Taub-Montemayor, T. E.; Min, K. J.; Chen, Z.; Bartlett, T.; Rankin, M. A. JH-III production, titers and degradation in relation to reproduction in male and female *Anthonomus grandis*. *J. Insect Physiol.* **2005**, *51*, 427−434.

(13) Kou, R.; Chou, S. Y.; Huang, Z. Y.; Yang, R. L. Juvenile hormone levels are increased in winners of cockroach fights. *Horm. Behav.* **2008**, *54*, 521−527.

(14) Chen, Z.; Linse, K. D.; Taub-Montemayor, T. E.; Rankin, M. A. Comparison of radioimmunoassay and liquid chromatography tandem mass spectrometry for determination of juvenile hormone titers. *Insect Biochem. Mol. Biol.* **2007**, *37*, 799−807.

(15) Min, K. J.; Jones, N.; Bordt, D. W.; Rankin, M. A. Increased juvenile hormone levels after long-duration flight in grasshopper *Melanoplus sanguinipes*. *J. Insect Physiol.* **2004**, *50*, 531−537.

(16) Westerlund, S. A.; Hoffmann, K. H. Rapid quantification of juvenile hormones and their metabolites in insect haemolymph by liquid chromatography−mass spectrometry (LC-MS). *Anal. Bioanal. Chem.* **2004**, *379*, 540−543.

(17) Cai, S. S.; Short, L. C.; Syage, J. A.; Potvin, M.; Curtis, J. M. Liquid chromatography−atmospheric pressure photoionization-mass spectrometry analysis of triacylglycerol lipids−Effects of mobile phases on sensitivity. *J. Chromatogr., A* **2007**, *1173*, 88−97.

(18) Cai, S. S.; Syage, S. A.; Hanold, K. A.; Balogh, M. P. Ultra performance liquid chromatography-atmospheric pressure photoionization-tandem mass spectrometry for high-sensitivity and high-throughput analysis of U.S. Environmental Protection Agency 16 priority pollutants polynuclear aromatic hydrocarbons. *Anal. Chem.* **2009**, *81*, 2123−2128.

(19) Takino, M.; Yamaguchi, K.; Nakahara, T. Determination of carbamate pesticide residues in vegetables and fruits by liquid chromatography−atmospheric pressure photoionization−mass spectrometry and atmospheric pressure chemical ionization−mass spectrometry. *J. Agric. Food Chem.* **2004**, *52*, 727−735.

(20) Matuszewski, B. K.; Constanzer, M. L.; Chavez-Eng, C. M. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal. Chem.* **2003**, *75*, 3019−3030.

(21) Chu, S.; Letcher, R. J. Analysis of fluorotelomer alcohols and perfluorinated sulfonamides in biotic samples by liquid chromatography-atmospheric pressure photoionization mass spectrometry. *J. Chromatogr., A* **2008**, *1215*, 92−99.

(22) Pérez-Hedo, M.; Goodman, W. G.; Schafellner, C.; Martini, A.; Sehnal, F.; Eizaguirre, M. Control of larval−pupal-adult molt in the moth *Sesamia nonagrioides* by juvenile hormone and ecdysteroids. *J. Insect Physiol.* **2011**, *57*, 602−607.

(23) Pérez-Hedo, M.; Eizaguirre, M.; Sehnal, F. Brain-independent development in the moth *Sesamia nonagrioides*. *J. Insect Physiol.* **2010**, *56*, 594−602.

(24) Miyazaki, M.; Mao, L. X.; Henderson, G.; Laine, R. A. Liquid chromatography−electrospray ionization-mass spectrometric quantitation of juvenile hormone III in whole body extracts of the Formosan subterranean termite. *J. Chromatogr., B* **2009**, *877*, 3175−3180.

(25) Food and Drug Administration. International Conference on Harmonisation [ICH]. Validation of analytical procedures: methodology, Q2B. *Fed. Regist.* **1997**, *62*, 27464−27467.

(26) Commission Decision 2002/657/EC. *Off. J. Eur. Communities* **2002**, *221*, 8−36.