JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Development of a HPLC/Tandem-MS Method for the Analysis of the Larvicides Methoprene, Hydroprene, and Kinoprene at Trace Levels Using Diels−Alder Derivatization

PAVEL A. ARONOV,[†] KATJA DETTMER,[†] JULIE A. CHRISTIANSEN,[‡] ANTHONY J. CORNEL,[‡] AND BRUCE D. HAMMOCK*,[†]

Department of Entomology, University of California at Davis, Davis, California 95616, and Mosquito Control Research Laboratory, Department of Entomology, University of California at Davis, 9240 South Riverbend Avenue, Parlier, California 93648

The invasion and subsequent spread of the mosquito-borne West Nile virus in the United States has resulted in increased use of methoprene. With the increased need for sensitive detection and monitoring of methoprene in the environment, an analytical LC/ESI−MS/MS method has been developed for the analysis of methoprene and two analogues, kinoprene and hydroprene, in water. To improve the ionization efficiency of the nonpolar analytes, a derivatization step with the Cookson-type reagent 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) was used. Derivatization improved the limit of detection 100-fold. For tandem MS analyses, limits of detection in environmental water samples (S/N = 3) are about 6 pg/mL for methoprene and 20 pg/mL for kinoprene and hydroprene, resulting in limits of quantification (S/N = 10) of 20 pg/mL for methoprene and 60 pg/mL for hydroprene and kinoprene extracted from 10 mL of water. This method was applied to measure methoprene concentrations in water samples from a treated site.

KEYWORDS: Methoprene; kinoprene; hydroprene; West Nile virus; ESI−MS; Cookson-type reagent

INTRODUCTION

Juvenile hormone (JH) analogues such as methoprene, hydroprene, and kinoprene are widely used larvicides for pest control because of their negligible toxicity to vertebrates and other nontarget organisms (*1*, *2*) and rapid degradation after application (*2*, *3*). There has been a recent increase in methoprene application to control mosquitoes because of the spread of the West Nile (WN) virus into the U.S.A. The presence of this virus, which is transmitted by numerous mosquito species, was first detected in 1999 in New York State. By August 2004, its presence was confirmed in all states except Washington, Montana, Hawaii (*4*), and Alaska. Methoprene is currently one of the few approved pesticides used to efficiently control aquatic stages of mosquitoes. Extensive methoprene use requires sensitive and robust analytical tools to monitor postapplication concentrations and presence.

Several analytical methods including GC/MS (*2*), GC/FID (*3*), and HPLC/UV (*5*) have been used for methoprene analysis. Linking liquid chromatography with mass spectrometry (LC/MS) often improves the selectivity and sensitivity that allows the simplification of the sample preparation and analysis. Methoprene analysis using electrospray ionization and single-quadrupole MS detection has already been reported by Wang et al. (*6*); however, because of the nonpolar nature of methoprene, its ionization efficiency in the electrospray process is low. In addition, single-quadrupole MS detection may not provide sufficient selectivity and sensitivity for reliable environmental sample analysis.

In the current study, an analytical method for methoprene detection and quantification is described. The method includes HPLC separation, electrospray ionization in positive mode, and tandem mass spectrometry using selected reaction monitoring.

However, electrospray ionization and tandem MS detection of methoprene lacks the sensitivity necessary for trace analysis in environmental samples because of its insufficient ionization efficiency in the electrospray process. Derivatization was, therefore, used in this work to enhance the sensitivity. Diels−Alder cycloaddition represents one way to make methoprene more readily ionizable. Methoprene contains a diene structure, which is able to undergo a 4+2 Diels−Alder cycloaddition with appropriate dienophiles at room temperature.

The Cookson-type reagent 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD), previously reported in vitamin D analysis (*7*, *8*), is used for analyte derivatization (**Figure 1**). PTAD readily reacts with the diene group of methoprene and other JH analogue pesticides (**Figure 1**). The derivative contains a proton-accepting amide group facilitating positive-mode electrospray ionization. In addition, the higher mass of the derivatized analytes allows

* To whom correspondence should be addressed: Department of Entomology, University of California, Davis, CA 95616. Telephone: (530) 752-7519. Fax: (530) 752-1537. E-mail: bdhammock@ucdavis.edu.
[†] Department of Entomology, University of California at Davis.
[‡] Mosquito Control Research Laboratory, Department of Entomology, University of California at Davis.

10.1021/jf0485842 CCC: $30.25
Published on Web 04/05/2005

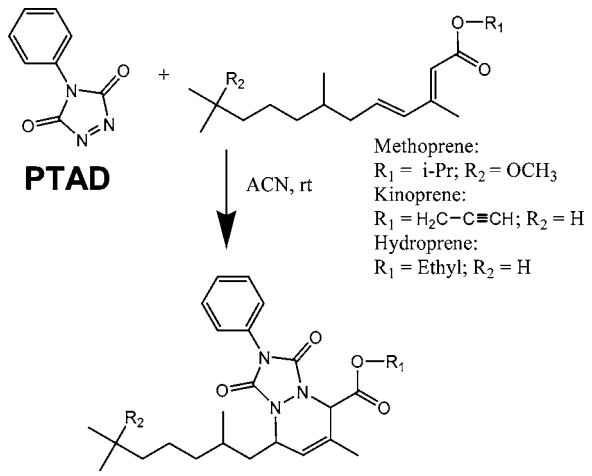

Figure 1. Derivatization reaction.

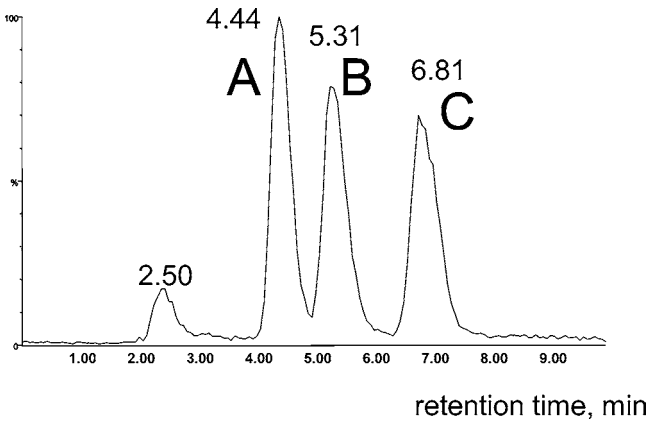

Figure 2. HPLC separation of a standard mixture under optimum conditions withMS detector (MRM mode) [5 ng/mL, 10 μL injected]. (A) Methoprene–PTAD. (B) Kinoprene–PTAD. (C) Hydroprene–PTAD.

better mass spectrometric analysis because of the lower background. In addition to the optimization of HPLC separation and mass spectrometric detection, a procedure for solid-phase extraction has been developed and applied to extract methoprene from water samples.

Resistance to methoprene had been reported in the mosquito *Ochlerotatus nigromaculis* (Ludlow) from other locations approximately 15 miles northeast of the field site in Riverdale (*9*), and a year later mosquito abatement personnel were concerned that resistance had spread despite their attempts to curtail it. Consequently, a field trial was conducted to determine methoprene susceptibility profiles of these mosquitoes and to evaluate the sensitivity of the newly developed method for methoprene analysis from water samples taken from this field pre- and post-treatment.

MATERIALS AND METHODS

Chemicals. (*S*)-Methoprene, (*S*)-kinoprene, and (*S*)-hydroprene were kindly provided by Welmark International (Dallas, TX). Stock solutions of the pesticides were prepared weekly in methanol and kept in the dark at +4 °C. PTAD was purchased from Fluka (Milwaukee, WI). Methanol, ethyl acetate, and acetonitrile used for extraction and HPLC separation, and sodium azide, were purchased from Fisher Scientific (Pittsburgh, PA). All solvents were HPLC-grade. Water solutions were prepared with deionized water (resistivity of 18.1 MΩ/cm).

At the field site, (Riverdale, Fresno County, CA) Altosand (Zoecon division of Wellmark International, Dallas, TX) with 5% *S*-methoprene was prepared and applied at maximum application rates according to the instructions of the manufacturer (*9*) when the mosquitoes were in late 3rd and early 4th stages.

Water Samples. Water samples were collected from 13 preselected sites prior to Altosand application and from the same 13 sites again immediately after application at 2 p.m. (day 1). Additional samples were collected at 2 p.m. each day for 4 days post-treatment. Altosand was applied at 8 of those 13 preselected sites and 5 sites were left untreated as controls. Individual samples were collected in 138 mL amber glass bottles (Fisher Scientific, Pittsburgh, PA) according to standard methodology (*10*) and preserved with 10% methanol and 0.02% sodium azide. Samples were kept at 4 °C prior to analysis.

Solid-Phase Extraction. Prior to extraction, 38 mL aliquots of water samples were removed for storage and the remaining 100 mL aliquots in the bottles were spiked with 10 μL of 44 μg/mL kinoprene in methanol. The bottles were shaken vigorously for 1 min, and 10 mL aliquots were taken for SPE extraction. SPE extraction was performed using Oasis HLB 3 cm^3 (60 mg) extraction cartridges (Waters, Milford, MA). Prior to sample application, the cartridges were activated with 2 mL of methanol followed by 2 mL of water. After sample extraction, the cartridges were washed with 3 mL of water and the analytes were eluted with 2 mL of ethyl acetate into 4 mL collecting glass vials (Fisher Scientific, Pittsburgh, PA). Each water sample was extracted in four replicates, and extracts were stored at −80 °C. The SPE method was shown to yield high recovery of the 3 analytes, but it was not optimized to minimize possible matrix effects.

Table 1. Mass Spectrometry Detection Parameters and Sensitivity Comparison

analyte	precursor ion (*m*/*z*)	production (*m*/*z*)	collision voltage (V)	calibration curve equation	R^2	sensitivity increase (fold)
methoprene	311	191	12	$y = 5.0675x + 416.67$	0.9778	
kinoprene	277	137	12	$y = 15.217x - 124.73$	0.9996	
hydroprene	267	109	21	$y = 16.811x + 5065.5$	0.9960	
methoprene–PTAD	454	242	21	$y = 6641.8x - 525.17$	0.9943	1310
kinoprene–PTAD	452	242	21	$y = 1555.2x + 1051.7$	0.9991	102
hydroprene–PTAD	442	242	21	$y = 1524.4x + 1567.8$	0.9920	91

Derivatization. Ethyl acetate extracts were evaporated under a gentle stream of nitrogen at room temperature, and 200 μL of 750 μg/mL PTAD in acetonitrile were added to the vials. The vials were vortexed for 1 min, and then 100 μL of the sample was transferred to 150 μL glass inserts (Waters, Milford, MA) in 2 mL amber HPLC vials (Fisher Scientific, Pittsburgh, PA). Samples were allowed to react at room temperature for 1 h after PTAD was added.

LC and MS Conditions. Chromatographic separation was performed using a Waters 2790 separation module (Waters, Milford, MA) equipped with a 150 × 2.00 mm Hypersil 3 μm C18-BD column (Phenomenex, Torrance, CA) held at 20 °C. A solvent system consisting of water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B) was used. The analytes were separated using a gradient program (0.2 mL/min) starting with a solvent composition of 80% solvent B, held for 2 min, and then ramped using a linear gradient for 8 min to 100% solvent B, held for 2 min (**Figure 2**). The injection volume was 10 μL. The samples were kept at 10 °C in the autosampler.

Analytes were detected by electrospray ionization in positive mode, tandem quadrupole mass spectrometry in multiple reaction monitoring mode (MRM) using a Quattro Ultima tandem quadrupole mass spectrometer (Micromass, Manchester, U.K.). Nitrogen gas flow rates were fixed with a cone gas flow of 125 L/h and a desolvation gas flow of 700 L/h. Electrospray ionization was performed in positive mode with a capillary voltage fixed at 3.00 kV and a cone voltage fixed at 30 V using a source temperature of 125 °C and a desolvation temperature of 350 °C. Capillary and cone voltage were optimized in an infusion experiment (data not shown). Argon was used as collision gas (2.3×10^{-3} Torr). Optimum collision voltages were determined experimentally by acquisition of product ion spectra (**Table 1**). These spectra were used to select a dominant product ion to set up the transition monitored in the MRM mode.

Quantification. Selected ion chromatograms were integrated using the QuantLynx module of the MassLynx 4.0 software (MicroMass, Manchester, U.K.). The methoprene concentration was normalized to the kinoprene concentration. Final methoprene concentration in water sample (C_{meth}^{env}) was calculated as

$$C_{meth}^{env} = C_{meth}^{exp} \frac{C_{kyn}^{theor}}{C_{kyn}^{exp}} D\omega$$

where D is a ratio of the original extracted water volume to final LC sample volume (concentration factor), ω is a dilution of original water sample with methanol (methanol dilution factor), C_{meth}^{exp} and C_{kyn}^{exp} are concentrations of methoprene and kinoprene experimentally found in a concentrated sample, and C_{kyn}^{theor} is the theoretical kinoprene concentration from the added internal standard in a concentrated sample. The values of D and ω were constant for all samples and equal 50 (10.0 mL/0.2 mL) for D and (138.0 mL/124.5 mL $\approx$ 1.1) for ω. Experimental concentrations of methoprene and kinoprene were quantified using external standard calibration. Calibration curves for both compounds contained five points from 0.3 to 30.0 ng/mL and were linear.

Mosquito Sampling and Susceptibility Assays. Before methoprene was applied to the pasture, 1st and 2nd stage larvae were collected and reared in the laboratory to the 4th stage for bioassays (*11*). In addition, to compare mortality observed in the laboratory assays, pupae were collected from several locations at the same pasture including the five pools of water from which water samples were taken for methoprene quantification.

RESULTS AND DISCUSSION

Derivatization Procedure. In a preliminary experiment, the derivatization efficiency and kinetics of the Diels−Alder reaction (**Figure 1**) were investigated by online monitoring of derivative formation using high-resolution time-of-flight mass spectrometry (TOF−MS, LCT, Micromass, Manchester, U.K.). The PTAD and pesticide concentrations were 166 μg/mL and 41.6 ng/mL (7000:1 molar ratio). The reaction mix was continiously infused in the TOF−MS detector, and $[M+H]^+$ ions of derivatized and nonderivatized pesticides were monitored. Peak heights of the ions were acquired every 10 min and averaged over 1 min intervals. Methoprene, kinoprene, and hydroprene were completely converted into the corresponding derivatives in about 1 h at room temperature (see Figure A in the Supporting Information). In methoprene, the diene is conjugated to the ester, and therefore, it is much less reactive than a diene lacking conjugation to an electron-withdrawing group. To prevent possible loss of PTAD because of the reaction with more reactive dienes present in water samples, the final PTAD concentration was increased to 750 μg/mL for derivatization of environmental samples. The increase of the PTAD concentration did not affect the noise level in the chromatograms (data not shown).

Optimization of the MS Parameters. To develop a specific and sensitive method for the analysis of methoprene, selective reaction monitoring was chosen as an operating mode for the tandem mass spectrometer. Optimum cone voltages for the formation of the precursor ions and preferable molecular ions $[M+H]^+$, during electrospray ionization, were determined by infusion of derivatized and nonderivatized analytes into the mass spectrometer. The derivatized analytes exhibited the highest ionization efficiency in positive mode, probably because of the nitrogen introduced in the molecule after derivatization (**Figure 3**). The nonderivatized analytes also were ionized in the positive mode and showed no ion formation in the negative mode. Methoprene−PTAD undergoes decomposition in the ionization source producing the very abundant decomposition ion $[M+H-32]^+$ caused by a loss of methanol (**Figure 3**). This ion was

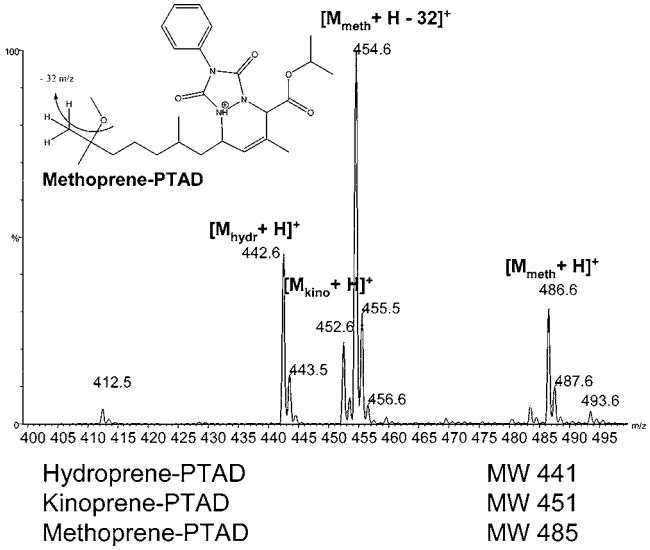

Figure 3. Full-scan spectrum of an analyte mix. Molecular masses of nonionized (nonprotonated) analytes are shown.

chosen as a precursor ion for methoprene−PTAD detection. Product ion spectra of nonderivatized and derivatized analytes using the molecular ions and $[M+H-32]^+$ for methoprene, respectively, as the precursor ion, were acquired (**Figure 4**). Fragmentation patterns of the derivatized analytes were similar (**Figure 4**). A characteristic ion with m/z 242 was found in all spectra, and the putative structure of the ion is shown in **Figure 4**. Similar ions were observed for PTAD derivatives of vitamin D_3 synthetic analogues in the MS^2 fragmentation experiment (8). Thus, fragmentation of methoprene and vitamin D derivatives does not involve retro Diels−Alder. This ion was chosen as a representative product ion for all PTAD derivatives in tandem MS experiments. The optimized detection parameters are shown in **Table 1**. The formation of a common product ion for PTAD derivatives could be used in the future to develop a tandem MS-based method to screen for compounds containing a diene structural unit.

Calibration Curve. Calibration curves were obtained for analytes based on chromatographic separation of the standards with detection conditions described above. For nonderivatized compounds, calibration curves were linear from 300 to 20 000 pg per compound injected onto the column. Derivatization resulted in about 3 orders of magnitude sensitivity increase for methoprene and 2 orders of magnitude sensitivity increase for kinoprene and hydroprene (**Table 1**). Higher sensitivity for methoprene is due to high abundance of its $[M+H-32]^+$ in-source decomposition ion, which was selected as a precursor ion. Calibration curves were linear in the range of 3−300 pg of analytes applied on the column. The highest sensitivity was obtained for methoprene−PTAD; however, it had also the highest variability, which resulted in the lowest R^2 values. This variability is probably due to a less reproducible decomposition of methoprene−PTAD in the ionization source.

Extraction Procedure. Surface water from Putah Creek was used to develop the SPE protocol. Water was spiked with known amounts of methoprene, kinoprene, and hydroprene and applied to the extraction cartridges. Volumes of 10, 20, and 30 mL of water containing 1, 2, and 3 ng of each analyte, respectively, were extracted. The cartridges were eluted twice with 2 mL of ethyl acetate. The experiments were performed in triplicates. The recovery experiments showed that more than 95% of the

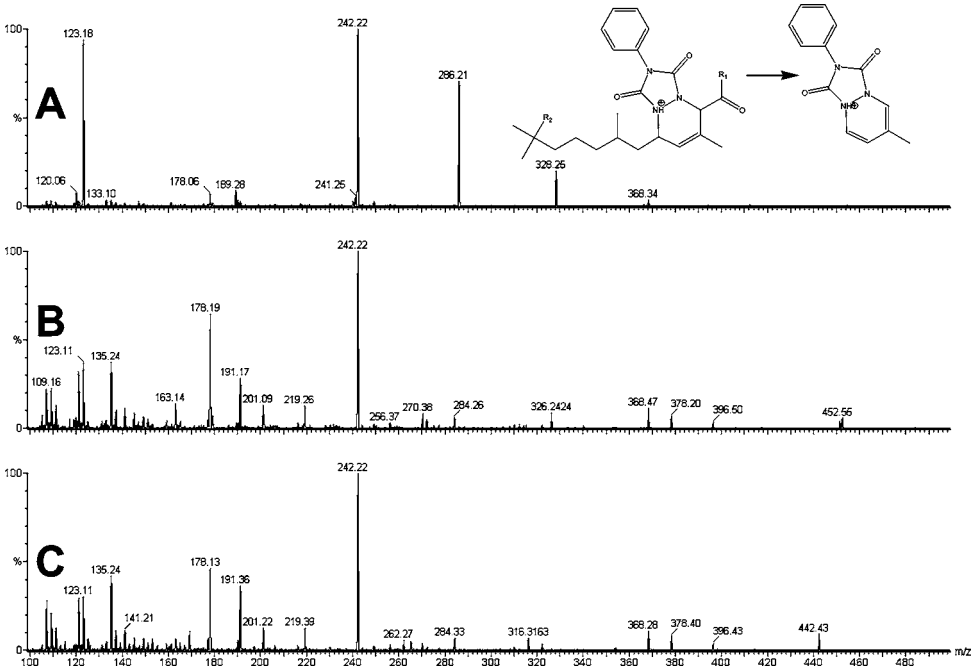

Figure 4. Daughter ion spectra. (A) Methoprene–PTAD. (B) Kinoprene–PTAD. (C) Hydroprene–PTAD. In spectrum A, putative fragmentation of the analytes and formation of the common product ion is shown. Charge position in the molecules is arbitrary.

relative amounts of the analytes were eluted in the first 2 mL of ethyl acetate (data not shown).

Sample concentration under a nitrogen blanket at room temperature and transfer of the analytes from collecting vials into HPLC vials resulted in absolute losses of 41 ± 15% methoprene, 62 ± 6% kinoprene, and 58 ± 10% hydroprene, respectively ($n = 3$). Addition of pentadecane as an analyte trap prior to ethyl acetate evaporation under nitrogen blanket and solvent evaporation with RC10.22 Speedvac concentrator (Jouan, Winchester, VA) did not improve analyte recovery significantly (data not shown). However, solvent evaporation in a Speedvac was 4 times faster and did not require nitrogen. Therefore, speedvac evaporation can be used as an alternative sample concentration technique.

Further investigation showed that analyte losses during transfer were probably due to absorption on the walls of glass vessels. To circumvent this problem, kinoprene was used as an internal standard for methoprene analysis because it is similar in structure and has the most similar retention properties on the reversed-phase column used in the experiments.

One of the major problems encountered in the analysis of methoprene is the susceptibility of the analyte to bio- and photodegradation as well as its adsorption at the glass walls of the sampling vessel. The influence of temperature, biodegradation, photodegradation, and adsorption was studied in a 3 day trial study to develop a protocol for handling of the samples. Amber glass bottles with the caps lined on the inside with aluminum foil were filled with Puta Creek water containing 0.02% sodium azide and 10% (volume) methanol. These trial samples were spiked with the mix of the analytes (100 pg/mL of each) and were kept at +4 °C in a refrigerator for 3 days. The influence of deviations from this protocol was studied; therefore, one bottle was kept at the strict protocol conditions, while others were kept with omission of several storage parameters (one bottle per experiment). On the third day, samples were extracted in triplicates as described above. As a control of recovery, a freshly prepared solution of analytes (100 pg/mL of each) in water with 10% (volume) methanol was used. The results are summarized in **Table 2**.

Samples must be stored at +4 °C in the dark prior to analysis. Sodium azide addition and amber glass bottles did not prevent analyte decomposition at room temperature and ambient light exposure. Aluminum foil lining the cap of the bottle significantly reduces the loss of analytes because of the prevention of adsorption to the plastic cap.

Analysis of Environmental Samples and Method Validation. A decrease in sensitivity was observed while running a sequence of 200 samples. This was assessed by a comparison of the chromatographic peak area of the internal standard (kinoprene) (see Figure B in the Supporting Information). Sensitivity decreased around 10-fold during analysis of the first 50 samples and then remained stable for the rest of the sequence. The loss of sensitivity was probably due to the accumulated residue on the sample cone and hexapoles.

For a conservative evaluation of the instrument performance, it is therefore practical to estimate the instrumental limits of detection (LOD) and quantification (LOQ) when the instrumental sensitivity is stable though moderate. Sample LOD and LOQ were estimated by determining minimal concentrations of calibration standards resulting in chromatographic peaks with a signal-to-noise ratio ≥ 3 (LOD), and 10 (LOQ), respectively, and divided by a factor of 50 because water samples were 50-fold concentrated. Signal-to-noise ratios were calculated as root-mean-squared values using MassLynx 4.0 software (MicroMass, Manchester, U.K.). Conservative estimates of LOD and LOQ are shown in **Table 3**. If the deterioration in signal could be prevented, the sensitivity of the method would be more than 5 times higher. It should be noted that signal deterioration does not affect quantification because the internal standard was used, which corrects this effect. The sensitivity of the reported method is 1 order of magnitude higher than that of the standard GC/MS-based method (*2*).

Although tandem mass spectrometers may not always be available in the laboratory because of their high costs, the reported derivatization approach can also be applied with other mass spectrometers using electrospray ionization including single-quadrupole instruments. A comparative study was performed to evaluate performance of a single-quadrupole instru-

Table 2. Influence of Various Factors on Sample Stability[a]

storage conditions	methoprene recovery (%) and SD	kinoprene recovery (%) and SD	hydroprene recovery (%) and SD
room temperature, scattered daylight, no methanol added, no sodium azide added, foil-lined cap, amber glass vial	nd[b]	nd	nd
+4 °C in the dark, methanol added, sodium azide added, no cap lining, amber glass vial	42.5 ± 6.7	71.1 ± 16.8	53.3 ± 8.7
+4 °C in the dark, methanol added, sodium azide added, foil-lined cap, clear glass vial	49.3 ± 19.0	104 ± 57	68.6 ± 26.2
+4 °C in the dark, no methanol added, sodium azide added, no cap lining, amber glass vial	61.8 ± 12.1	88.9 ± 10.2	80.0 ± 9.9
+4 °C in the dark, methanol added, sodium azide added, no cap lining, amber glass vial	65.7 ± 16.0	109 ± 37	89.5 ± 23.1

[a] Recoveries were normalized to the recovery of standard 10% methanol solution prepared immediately before the extraction. [b] nd = not detected.

Table 3. Method Limit of Detection and Quantification

	sample LOD (pg/mL)	sample LOQ (pg/mL)
methoprene–PTAD	6	20
kinoprene–PTAD	20	60
hydroprene–PTAD	20	60

Table 4. Comparison of Single Quadrupole and Tandem Quadrupole Performance[a]

		MRM mode	SIR mode
control	peak area	182 300	822 100
	signal-to-noise ratio	6330	250
matrix ($n = 3$)	peak area	145 200	497 800
	signal-to-noise ratio	1360	90

[a] Derivatized methoprene was dissolved in pure solvent (control) and extracted matrix, and 100 pg was injected on the column. For the matrix, averages of triplicate are shown.

ment. Methoprene standards were dissolved in pure acetonitrile as well as in the extract from Putah Creek water matrix (extracted as described above). These two groups of samples were analyzed in MRM and SIR (single-ion recording) modes using a Quattro Ultima tandem quadrupole mass spectrometer (Micromass, Manchester, U.K.). The latter mode is analogous to SIR acquisition using a single-quadrupole instrument. **Table 4** shows the results for methoprene−PTAD at 10 ng/mL concentration (100 pg injected on the column). Signal-to-noise ratios and peak areas were calculated using MassLynx 4.0 software as described above (MicroMass, Manchester, U.K.). Although the absolute value of analyte signal is higher in SIR mode, MRM mode provided a superior signal-to-noise ratio and lower background level, especially if the analyte is dissolved in the complex matrix. With sample preconcentration using a SPE column, a single-quadrupole instruments may provide reasonable sensitivity for derivatized analytes.

Derivatization destroys the conjugated diene chromophore group of the analytes (**Figure 6**); therefore, they are not optimum for HPLC with UV detection. However, a PTAD analogue with a chromophore group could be synthesized to improve method sensitivity. Fluorescent analogues of PTAD such as the commercially available 4-[4-(6-methoxy-2-benzoxazolyl)phenyl]-1,2,4-triazolidine-3,5-dione (MBOTAD) can be used for detection of methoprene derivatives by HPLC using a fluorescence detector (*12*).

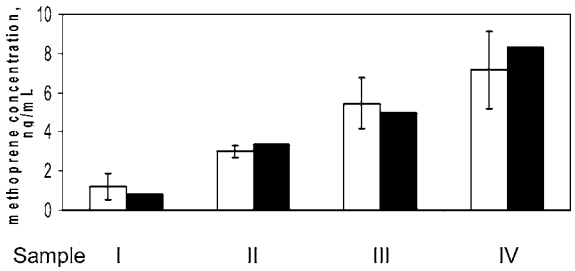

Figure 5. Method validation. Analysis of environmental water samples spiked with known concentrations of methoprene and kinoprene (internal standard). White bars, experimental values; black bars, theoretical values. Error bars represent confidence intervals at a 95% probability level.

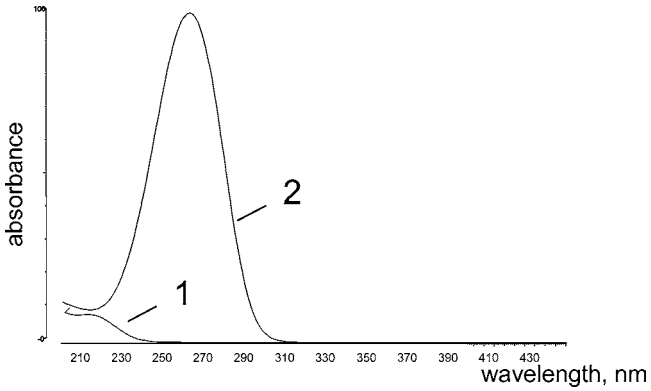

Figure 6. UV spectra of derivatized and nonderivatized methoprene. (1) Derivatized methoprene. (2) Nonderivatized methoprene. Spectra were acquired using Waters 2996 Photodiode Array Detector (Waters, Milford, MA).

Operationally significant tolerance (resistance) to methoprene was detected in *O. nigromaculis* mosquitoes from the Riverdale site. Mortality of only 26.1% was recorded from field-collected pupae, and from susceptibility assays, LD_{30}, LD_{50}, and LD_{90} of 1.14, 15.9, and 9.9 ng/mL were recorded, respectively. Probit analysis on the susceptibility assays produced an LD_{26} confidence interval (95%) of 0.07−6.83 ng/mL. Consequently, we would expect that the concentration of methoprene in the field 24 h after application when larvae were in the 4th stage (stage for methoprene action) should fall within this range. The methoprene concentration determined in environmental water samples from the field experiment are shown in **Table 5**. Each

Table 5. Results of Methoprene Concentration Measurements at Eight Sites of the Flooded Pasture Treated with Altosand

	methoprene concentration (ng/mL) and SD				
site	day 1	day 2	day 3	day 4	day 5
1	2.36 ± 0.22	0.224 ± 0.047	0.273 ± 0.048	0.106 ± 0.014	0.345 ± 0.042
2	0.116 ± 0.018	0.198 ± 0.010	0.115 ± 0.014	0.064 ± 0.010	0.226 ± 0.037
3	0.701 ± 0.066	0.153 ± 0.007	0.108 ± 0.079	0.055 ± 0.006	0.166 ± 0.019
4	1.36 ± 0.04	0.412 ± 0.042	0.184 ± 0.038	0.178 ± 0.022	0.169 ± 0.022
5	1.32 ± 0.07	0.094 ± 0.010	2.26 ± 0.27	0.057 ± 0.002	2.59 ± 0.12
6	0.469 ± 0.019	0.125 ± 0.005	nd[a]	0.054 ± 0.007	0.054 ± 0.006
7	2.62 ± 0.17	0.040 ± 0.005	0.299 ± 0.000	nd	0.050 ± 0.009
8	0.469 ± 0.019	0.090 ± 0.009	2.071[b]	nd	0.032 ± 0.006

[a] nd = not detected. [b] $N = 2$.

water sample was analyzed in four replicates. The reproducibility of the analysis was acceptable with average relative standard deviations of 12%. Methoprene was not detected in the samples collected before Altosand application and in the samples from five control sites. The rapid decline in methoprene concentration after Altosand application was expected because this formulation is not designed to have residual activity and methoprene is known to rapidly degrade under direct sunlight, high temperatures, and "microbially" rich waters (*3*, *13*), characteristic of conditions in California animal pastures.

Methoprene concentrations although variable from site to site all fell within the predicted range (0.07−6.83 ng/mL), which suggests that our susceptibility assays show correlations with field situations. One would realistically expect variations in methoprene concentrations across an irrigated pasture. Uniform applications of Altosand (numbers of sand granules falling per square area) are difficult because of the pure mechanics of maintaining a constant speed on rough terrain and maintaining constant numbers of sand granules flung out from the vehicle-mounted seeder. Furthermore, because pastures are not uniformly flat and have uneven vegetation coverage, flood-irrigated water immediately unevenly evaporates because of variable shading and uneven seepage through the substrate. Consequently, pools of variable depth form, and methoprene-contaminated water is unevenly drawn into areas of lowest depression (deeper pools) within the first 24 h after flooding.

Control environmental samples from five nontreated sites where methoprene was not detected were spiked in a blind fashion with methoprene of known concentrations in a range from 0.83 to 8.33 ng/mL and subjected to the same analytical protocol. Spiked methoprene concentrations are within the confidence limit at a 95% probability level (**Figure 5**).

Derivatization of methoprene, kinoprene, and hydroprene with the Cookson-type reagent PTAD significantly improved the sensitivity of the analytical method based on electrospray ionization in positive mode. The high sensitivity allows us the use of small sample volumes (10 mL) for the analysis of evanescent JH analogue larvicides in environmental water samples and can be used as an alternative to the previously reported high-volume liquid−liquid extraction method (*2*).

ACKNOWLEDGMENT

We thank Kathleen Thornton (Wellmark International, TX) for providing the pesticide standards, Steve Mulligan III (manager of the Consolidated Mosquito Abatement District) for methoprene field application, as well as George Lindahl (Wellmark International, TX), John Newman, Paul Jones, and Theresa Pedersen (Department of Entomology, University of California at Davis) for the valuable discussions.

Supporting Information Available: Derivatization kinetics and loss of instrument sensitivity in Figures A and B, respectively. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Degitz, S. J.; Durhan, E. J.; Tietge, J. E.; Kosian, P. A.; Holcombe, G. W.; Ankley, G. T. Developmental toxicity of methoprene and several degradation products in *Xenopus laevis*. *Aquat. Toxicol*. **2003**, *64*, 97−105.

(2) Henrick, C. A.; Ko, J.; Nguyen, J.; Burleson, J.; Lindahl, G.; Van Gundy, D.; Edge, J. M. Investigation of the relationship between *S*-methoprene and deformities in anurans. *J. Am. Mosq. Control Assoc*. **2002**, *18*, 214−221.

(3) Ross, D. H.; Judy, D.; Jacobson, B.; Howell, R. Methoprene concentration in freshwater microcosms treated with sustained-release Altosid formulations. *J. Am. Mosq. Control Assoc*. **1994**, *10*, 202−209.

(4) *Morbidity and Mortality Weekly Report*; Centers for Disease Control and Prevention; Vol. 53, pp 742−743.

(5) Abu-Qare, A. W.; Abou-Donia, M. B. A solid-phase extraction reversed-phase HPLC method for the simultaneous determination of methoprene, permethrin, and selected metabolites in rat plasma and urine. *Biomed. Chromatogr*. **2001**, *5*, 464−470.

(6) Wang, I.-S.; Moorman, R.; Burleson, J. Isocratic reversed-phase liquid chromatographic method for the simultaneous determination of (*S*)-methoprene, MGK264, piperonyl butoxide, sumithrin, and permethrin in pesticide formulation. *J. Chromatogr., A* **2003**, *983*, 145−152.

(7) Higashi, T.; Miura, K.; Kikuchi, R.; Shimada, K.; Hiyamizu, H.; Ooi, H.; Iwabuchi, Y.; Hatakeyama, S.; Kubodera, N. Characterization of new conjugated metabolites in bile of rats administered 24,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3. *Steroids* **2000**, *65*, 281−294.

(8) Weiskopf, A. S.; Vouros, P.; Cunniff, J.; Binderup, E.; Bjoerkling, F.; Binderup, L.; White, W.-C.; Posner, G. H. Examination of structurally selective derivatization of vitamin D_3 analogues by electrospray mass spectrometry. *J. Mass Spectrom*. **2001**, *36*, 71−78.

(9) Cornel, A. J.; Stanich, M. A.; Farley, D.; Mulligan, F. S.; Byde, G. Methoprene tolerance in *Aedes nigromaculis* in Fresno County, California. *J. Am. Mosq. Control Assoc*. **2000**, *16*, 223−228.

(10) *Guide to Sampling Air, Water, Soil, and Vegetation for Chemical Analysis*; State of California, Environmental Protection Agency, Department of Pesticide Regulation, Environmental Monitoring and Pest Management Branch, Environmental Hazards Assessment Program: Sacramento, CA, 1994; pp 7−10.

(11) Cornel, A. J.; Stanich, M. A.; McAbee, R. D.; Mulligan, F. S. High level methoprene resistance in the mosquito *Ochlerotatus*

nigromaculis (Ludlow) in central California. *Pest Manage. Sci.* **2002**, *58*, 791−798.

(12) Shimada K.; Mizuguchi T. Sensitive and stable Cookson-type reagent for derivatization of conjugated dienes for high performance liquid chromatography with fluorescence detection. *J. Chromatogr.* **1992**, *606*, 133−135.

(13) Schaeffer, C. H.; Dupras, E. F. Insect development inhibitors. 4. Persistence of ZR-515 in water. *J. Econ. Entomol.* **1973**, *66*, 923−925.

Received for review August 26, 2004. Revised manuscript received February 24, 2005. Accepted March 3, 2005. The financial support by the National Institute of Environmental Health Sciences, Grants R37 ES02710, P42 ES04699, 1 P01 ES11269, and U01 AI058267, USDA Competitive Research Grant 2003-35302-13499, and the German Research Foundation are gratefully acknowledged.

JF0485842