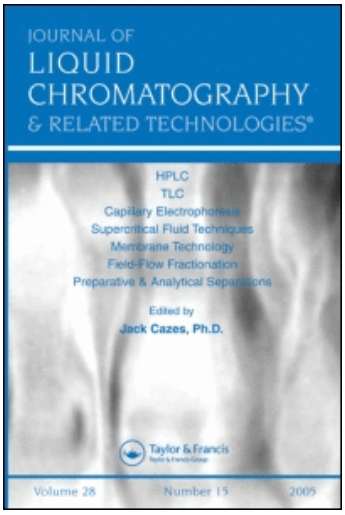

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Determination of Methoprene in Water Samples by High Performance Liquid Chromatography

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DETERMINATION OF METHOPRENE IN WATER SAMPLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A new high-performance liquid-chromatography method has been developed that can determine low concentrations of methoprene in water samples. The method allows for reliable detection of concentrations between 0.005 µg/mL and 0.5 µg/mL. The detection limit for 5-mL samples is 2.5 ng/mL at 255 nm. The standard curve is linear over the entire concentration range. The method was used to do a stability study on aqueous methoprene samples stored at room temperature and under refrigeration. The study shows that aqueous methoprene samples are stable when stored at 4°C for at least three weeks.

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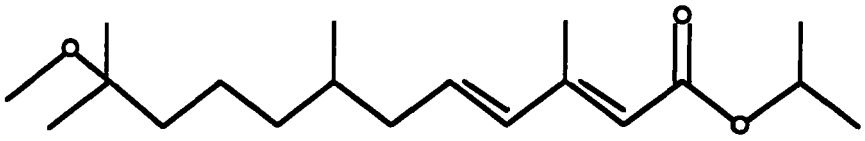
INTRODUCTION

Methoprene, a juvenile hormone analogue that can function as an insect growth regulator, is registered under the trade name Altosid (Zoecon Corporation). A potent and selective larvicide, methoprene (isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate, Figure 1) is toxic to insects, causing interference in the metamorphosis of the larva. Methoprene has been shown to be effective against mosquito larvae (1) and the larvae of Cyclops vernalis (2), also referred to as cyclopoid copepods. Dracunculiasis is a disease acquired by ingesting water containing cyclopoid copepods that are infected with the third-stage larva of the parasite Dracunculus medinensis. The World Health Organization has concluded that methoprene is harmless to humans at a level of 1 ppm in drinking water (3). Future studies of the effectiveness of methoprene as a larvicide to treat parasite-infested waters, will require a reliable quantitative method to determine the level of this insect growth regulator in water.

Other analytical methods for detecting methoprene have been reported. A gas chromatographic method (4), a

*The use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.



Methoprene
$C_{19}H_{34}O_3$

Figure 1. The structural formula of methoprene.

high-performance liquid chromatographic (HPLC) method (5), and an infrared spectroscopic method (6) have been reported. None of these were applied to water samples.

This paper descibes a reliable analytical method for extracting and quantitating methoprene in 5-mL water samples. HPLC with a reverse-phase column (C-18) and an ultraviolet detector set at 255 nm is used to detect methoprene in water samples with concentrations from 0.005 to 0.5 µg/mL.

EXPERIMENTAL

Chemicals

All organic solvents were of HPLC grade. Purified water was supplied from a filter purification system having both ion-exchange and carbon filters. The analytical standard methoprene (97.3% purity) was supplied

by Zoecon Corporation (Palo Alto, California). The internal standard, dipentyl phthalate, technical grade, was obtained from Eastman Kodak Co. (Rochester, N.Y.).

Equipment

All samples were analyzed on a Beckman 344 Gradient Liquid Chromatograph, equipped with an Altex 210A sample injector valve, an LDC/Milton Roy Spectromonitor D variable wavelength detector set at 255 nm, and a SpectraPhysics SP4270 integrator. The analysis used a Beckman Ultrasphere XL-ODS column, 4.6mm x 70mm, packed with 3-um particles; flow rate was 1.0 mL per minute at room temperature.

Preparation of Standard Solutions

Methoprene standard stock solution A, 9.016 µg/mL in CH_3CN, is prepared by making a 1:5 dilution from a more concentrated stock solution, 45.08 µg/mL in CH_3CN (prepared from analytical standard concentrate). The methoprene standard stock solution B, 0.9016 µg/mL in CH_3CN, is prepared by making a 1:10 dilution of the 9.016 µg/mL stock. The stock solutions are needed to prepare the working 5-mL standard solutions. Methoprene standards (5 mL) with concentrations of 0.005049 µg/mL,

0.01009 µg/mL, and 0.05049 µg/mL were prepared by adding 28 µL, 56 µL, and 280 µL, respectively, of stock B to 5 mL of H_2O. Methoprene standards (5 mL) with concentrations of 0.1009 µg/mL, 0.2525 µg/mL, and 0.5049 µg/mL were prepared by adding 56 µL, 140 µL, 280 µL, respectively, of stock A to 5 mL of H_2O. These standards must be prepared fresh in the same tube from which they are extracted because large volumes of aqueous stock solutions containing methoprene do not store well over short periods.

The internal standard solution of dipentyl phthalate was prepared directly from a stock solution of 2.27 mg/mL in CH_3CN by taking 1 milliliter of the stock and diluting to 100 mL with CH_3CN to give a working solution of 22.7 µg/mL in CH_3CN.

Analysis of Water Samples

Five milliliters of water was added directly into clean, dry, 15-mL glass centrifuge tubes. To each sample tube (including standards), 100 µL of internal standard solution (22.7 µg/mL dipentyl phthalate) was added. A 3-mL volume of methyl tert-butyl (MTBE) was added, and the tubes were vortexed for 1 minute. The tubes were centrifuged for 10 minutes at 2500 g to separate layers.

The MTBE layer was transferred to a clean 15-mL centrifuge tube and evaporated under N_2 in a warm (45°C) water bath. The dry residue was dissolved in 100 µL of mobile phase (90% CH_3CN/H_2O) by vortex mixing for 30 seconds. Standards, prepared as described above in glass centrifuge tubes, were extracted in the same manner as the samples.

Mobile phase was prepared by premixing 270 mL of CH_3CN and 30 mL of water, then filtering to degas. The 20-µL sample loop on the injector was overfilled by adding at least 30 µL. After each injection of standard or sample, the syringe was rinsed three times with CH_3CN to avoid contamination during subsequent injections.

A typical chromatogram of an aqueous methoprene sample is shown in Figure 2. The area count ratio of methoprene/ internal standard was calculated for each standard. These ratios and concentrations of the standard methoprene samples were used in a linear least-squares program to calculate the concentration of methoprene in the unknown water samples.

Sample Stability Study

The stability of methoprene solutions stored at room temperature (25°C) and refrigerated temperatures (4°C)was investigated. Eighteen samples of each

Figure 2. Representative chromatographic traces showing: A, extract of blank water sample showing only the internal standard, dipentyl phthalate, and B, extract of water sample spiked with 0.1 µg/mL methoprene.

concentration (blank, 0.05049 0.1009, 0.2525, 0.5049 µg/mL) were prepared in the same manner as previously described for standards. On day 0, a set containing duplicate samples of each concentration was extracted and analyzed. Additionally, a set of samples (8 tubes of each

concentration) was stored at room temperature; another set was stored at refrigerator temperatures (4°C). Duplicate samples of each concentration under both types of storage conditions were analyzed by the method described above on days 3, 7, 14, and 21.

RESULTS AND DISCUSSION

Analytical Method

The methoprene stock solutions in acetonitrile are stable for long periods when stored at 4°C. Standard curves were best when the aqueous standards were prepared fresh daily in the same glass tubes in which the extraction step was performed.

The analytical method shows good precision and reproducibility in the extraction process for methoprene. Table 1A shows the within-day precision of four 0.1009 µg/mL standards (RSD=4%), and Table 1B shows between-day precision of five 0.2525 µg/mL samples.

Storage Stability Study

Aqueous samples (5 mL) of varying concentration of methoprene were stored in duplicate at room temperature or under refrigeration. These samples were reasonably stable over a three-week period under refrigeration. The first

TABLE 1

Within-Day Precision of the Method

A)

Exact Sample Concentration	Calculated Concentration	Relative standard Deviation
0.1009 µg/mL	0.0939 µg/mL	4.0%
0.1009 µg/mL	0.0916 µg/mL	
0.1009 µg/mL	0.0971 µg/mL	
0.1009 µg/mL	0.0984 µg/mL	
	avg. = 0.0953	

Between-Day Precision of Method

B)

Exact Sample Concentration	Calculated Concentrations for Samples Prepared on Different Days	Relative standard Deviation
0.2525 µg/mL	0.2730 µg/mL	6.0%
0.2525 µg/mL	0.2340 µg/mL	
0.2525 µg/mL	0.2485 µg/mL	
0.2525 µg/mL	0.2627 µg/mL	
0.2525 µg/mL	0.2414 µg/mL	
	avg. = 0.2519	

definite signs of change of concentrations could be detected on day 7 at room temperatures (Table 2A), and on day 14 under refrigerated conditions (Table 2B). The overall change in levels of methoprene between samples of the same concentration was greater for those samples left at room temperature. The precision for those stored under refrigeration was acceptable (Table 2A and B).

TABLE 2

A) Stability of Methoprene Solutions (µg/mL) Stored at Room Temperature and Normal Lighting

Concentration	Day 0	Day 3	Day 7	Day 14	Day 21	% Change
Control	0	0	0	0	0	0%
.05049	0.0479	0.0464	0.0434	0.0551	0.0348	27%
.1009	0.1079	0.1157	0.0799	0.0991	0.0791	27%
.2525	0.2594	0.2392	0.2545	0.2375	0.1881	27%
.5049	0.5007	0.5095	0.5096	0.4574	0.4081	18%

B) Stability of Methoprene Solutions (µg/mL) Stored Under Refrigeration

Concentration	Day 0	Day 3	Day 7	Day 14	Day 21	% Change
Control	0	0	0	0	0	0%
.05049	0.0479	0.0491	0.0481	0.0468	0.0447	7%
.1009	0.1079	0.1094	0.1172	0.1093	0.1049	3%
.2525	0.2594	0.2534	0.2610	0.2552	0.2310	11%
.5049	0.5007	0.5319	0.5291	0.4766	0.4256	15%

CONCLUSION

The findings presented herein suggest that the HPLC method of analysis for aqueous methoprene samples is reliable and precise. The storage stability study shows that methoprene stored in an aqueous solution is relatively stable for three weeks under refrigeration.

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