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Determination of Acequinocyl and Hydroxyacequinocyl on Fruits and Vegetables by HPLC-DAD

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A method for determining residues of the new reduced-risk pesticide acequinocyl and its deacetylated derivative hydroxyacequinocyl on fruits and vegetables (grapes, lemons, pears, and tomatoes) by HPLC is described. The pesticides were extracted from the fruits and vegetables with hexane and ethyl acetate solution (1:1, v/v), determined by HPLC-DAD at 250 nm and confirmed by LC/MS. No cleanup was necessary. This method is characterized by recoveries (0.01−4 mg/kg) > 77%, while the coefficient of variation was determined to be less than 11%. The limit of quantitation for both acequinocyl and hydroxyacequinocyl was 0.01 mg/kg for all matrixes.

KEYWORDS: HPLC-DAD; acequinocyl; hydroxyacequinocyl; LC/ESI-MS

INTRODUCTION

Acequinocyl (3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate) (**Figure 1**) is an acaricide with contact action belonging to naphthoquinones used for the control of several species of mites in agricultural crops and ornamentals. It has been described by Kinoshita et al. and Wakasa and Watanabe (*1, 2*). The activity of this pesticide is due to its hydrolyzed derivative hydroxyacequinocyl (2-hydroxy-3-dodecyl-1,4-naphthoquinone) (**Figure 1**), which inhibits complex III (bc1 complex) binding at the Q_0 center and blocks cellular respiration (*3*). Because it acts at the complex III stage, it can be used to control mite populations which are resistant to other miticides. Acequinocyl shows a low level of toxicity (LD_{50} for rats of >5000 mg/kg of body weight), and it is considered as a reduced-risk pesticide. This product is registered in Japan, the U.S., Korea, and Taiwan for many crops, and field trials are conducted in Europe. The maximum residue limits (MRLs) are set as the sum of acequinocyl and its metabolite hydroxyacequinocyl. To our knowledge no analytical method is reported in the literature to determine acequinocyl residues in crops. In this paper an HPLC-DAD method to determine acequinocyl and its metabolite hydroxyacequinocyl residues in fruits and vegetables is described.

MATERIAL AND METHODS

Chemicals. Ethyl acetate, acetonitrile, and hexane were HPLC grade (Merck, Milan, Italy). Phosphoric acid (Carlo Erba, Milan, Italy) and trifluoroacetic acid (Sigma Aldrich, Steinheim, Germany) were 99% pure. Water was distilled and filtered through a Milli-Q apparatus (Millipore, Milan, Italy) before use. Acequinocyl (98% purity) and hydroxyacequinocyl (99% purity) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Stock standard solutions of the pesticides (ca. 1000 mg/L each) were prepared in hexane and stored in amber vials. Working standard solutions of the pesticides were prepared daily by diluting with the mobile phase (acetonitrile−aqueous 0.1% phosphoric acid, 90:10, v/v).

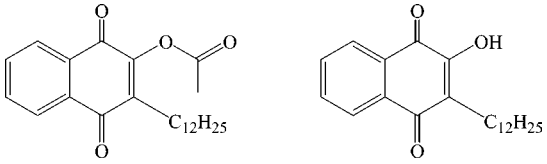

Figure 1. Structures of acequinocyl and hydroxyacequinocyl.

Instrumentation and Sample Analysis. *HPLC-DAD Analysis.* An Agilent Technologies (Waldbronn, Germany) model 1100 liquid chromatograph was used, fitted with a diode array detector (DAD), model UV6000LP (Thermo Quest, San Josè, CA). A Waters Spherisorb S5 ODS2 (250 × 4.6 mm, 5 μm) (Milford, MA) column was employed. Isocratic elution was with acetonitrile−aqueous 0.1% phosphoric acid (90:10, v/v) for 18 min. The sample injection volume was 100 μL, the flow rate was 1 mL/min, and the column was maintained at 20 °C. Quantitative analyses involved peak area comparisons with synthetic standards and absorbance measurements at 250 nm.

LC/MS Analysis. An HPLC system (Shimadzu, Milan, Italy) equipped with an SPD11 Avp DAD detector, an SIL 11 AD vp autoinjector, and an LC 10 AD binary pump coupled on line with an MS2010 mass spectrometer (Shimadzu, Milan, Italy) was used. UV and MS data were acquired and processed using Shimadzu "LCMS solution" software. Isocratic elution was with acetonitrile−aqueous 0.1% trifluoroacetic acid (99%) (90:10, v/v) for 30 min. The column used was a 150 × 2.1 i.d. 3.5 μm Waters Symmetry C18. The injection volume was 20 μL, and the flow rate was 0.4 mL/min. The ESI-MS interface was operated in the positive mode: ESI source probe 245 °C, CDL 245 °C, block at 230 °C, flow gas (N_2) at 4.5 mL/min, probe voltage 4.5 kV, scan 150−650 amu.

Extraction Procedure from Fruits. The samples were chopped and homogenized by a blender (Malavasi, Bologna, Italy), and 5 g of well-mixed chopped grapes, lemons, pears, and tomatoes was weighed into

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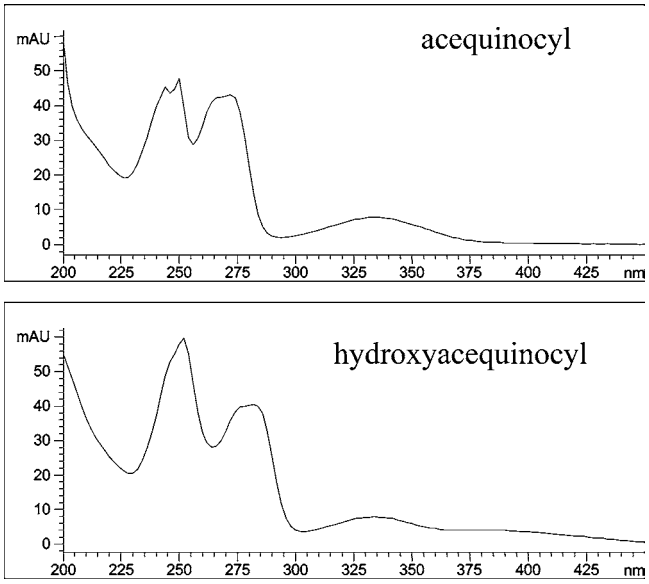

Figure 2. UV spectra of acequinocyl and hydroxyacequinocyl.

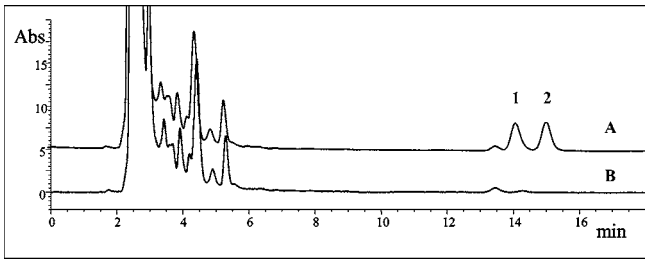

Figure 3. Chromatograms of extracts of tomatoes obtained under the operating conditions described in the text: (**A**) fortified sample at 0.2 mg/kg hydroxyacequinocyl (1) and acequinocyl (2), (**B**) control.

a 40 mL screw-capped tube, and 10 mL of a hexane/ethyl acetate (1:1, v/v) solution was added. The tube was agitated for 15 min in a rotary shaker, and 1 mL of the mixture was dried under a gentle nitrogen stream and dissolved in 0.5 mL of the mobile phase for HPLC analysis.

Recovery Assays. A 50 μL aliquot of pesticide solution at the desired concentration was added to each 5 g sample of untreated grapes, lemons, pears, and tomatoes. The fortification levels used were 0.01, 0.04, 0.10, 0.20, 1.0, and 4.0 mg/kg. The samples were allowed to settle for 30 min prior to extraction. They were later processed according to the above extraction procedure. Four replicates of each matrix were analyzed.

RESULTS AND DISCUSSION

As expected, the spectra of the two compounds were very similar, because their structures differed only in the presence of an acetyl instead of a hydroxy group in position 2 of the naphthoquinone moiety, and showed a maximum absorbance at 250 nm (**Figure 2**). For recovery determination the wavelength of maximum absorbance at 250 nm was used. The separation of acequinocyl and hydroxyacequinocyl (**Figure 3**) was obtained by isocratic analysis with acetonitrile−aqueous 0.1% phosphoric acid (90:10, v/v). Retention times for acequinocyl and hydroxyacequinocyl were 15.2 and 14.2 min, respectively.

Standard calibration curves of acequinocyl and hydroxyacequinocyl were constructed by plotting analyte concentrations against peak areas. Good linearity was achieved between 0.01 and 4 mg/kg with correlation coefficients between 0.9995 and 0.9998.

Table 1. Recoveries (% ± RSD) of Acequinocyl and Hydroxyacequinocyl on Fruits

crop	fortification level (mg/kg)	acequinocyl	hydroxyacequinocyl
pears	0.01	86 ± 9	83 ± 1
	0.04	95 ± 2	89 ± 5
	0.1	77 ± 1	95 ± 10
	0.2	81 ± 2	108 ± 6
	1	101 ± 1	94 ± 3
	4	95 ± 1	89 ± 11
lemons	0.01	84 ± 6	85 ± 5
	0.04	109 ± 2	85 ± 3
	0.1	109 ± 6	93 ± 5
	0.2	86 ± 6	80 ± 1
	1	110 ± 5	90 ± 2
	4	82 ± 2	81 ± 3
tomatoes	0.01	96 ± 4	109 ± 2
	0.04	87 ± 4	99 ± 9
	0.1	98 ± 1	92 ± 4
	0.2	92 ± 11	90 ± 3
	1	86 ± 4	89 ± 7
	4	88 ± 11	93 ± 4
grapes	0.01	89 ± 1	106 ± 11
	0.04	77 ± 6	77 ± 3
	0.1	104 ± 7	93 ± 5
	0.2	82 ± 8	94 ± 2
	1	86 ± 4	100 ± 4
	4	93 ± 1	95 ± 3

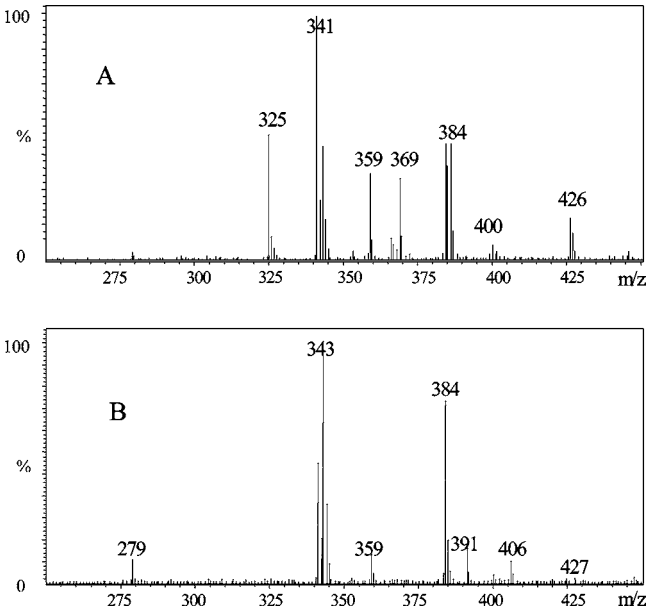

Figure 4. LC/ESI-MS spectra of acequinocyl (**A**) and hydroxyacequinocyl (**B**) obtained at 4.5 kV.

The pesticide extraction was performed with a hexane/ethyl acetate (1:1, v/v) solution. No cleanup was necessary because no interfering peaks were present. The recovery data are presented in **Table 1**. Recoveries ranged from 77% to 110%, with coefficients of variation between 1% and 11%. According to Thier and Zeumer (*4*), the limit of quantitation was 0.01 mg/kg for both acequinocyl and hydroxyacequinocyl. These low detection limits were obtained as the result of the high sensitivity of the detector, which was due to a cell path length of 50 mm. Utilizing the DAD made it possible to know the sample spectra and the peak purity and confirm the active ingredients by overlapping the sample spectra with those of the standards. A confirmation assay was performed by HPLC/MS using the conditions described above. The analysis was performed in the ESI mode, obtaining the following fragments: *m*/*z* 384 $[M]^+$, *m*/*z* 426 $[M + H + CH_3CN]^+$, *m*/*z* 369 $[M - CH_3]^+$, *m*/*z* 341

$[M - COCH_3]^+$, and *m*/*z* 325 $[M - OCOCH_3]^+$ for acequinocyl and *m*/*z* 343 $[M + H]^+$, *m*/*z* 384 $[M + H + CH_3CN]^+$, and *m*/*z* 406 $[M + CH_3CN + Na]^+$ for hydroxyacequinocyl (**Figure 4**).

The described method is simple and rapid and can be used for routine analysis to determine pesticide residues in food.

ABBREVIATIONS USED

LC/MS, liquid chromatography/mass spectrometry; ESI, electrospray ionization.

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