RESIDUES AND TRACE ELEMENTS

Liquid Chromatographic Determination of Five Benzoylurea Insecticides in Fruit and Vegetables

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A liquid chromatographic (LC) method was developed to determine 5 benzoylureas—diflubenzuron, hexaflumuron, teflubenzuron, flufenozuron, and lufenuron—in peppers, tomatoes, eggplants, cucumbers, and oranges. Preparation of samples involve extraction with acetone and partitioning into dichloromethane–petroleum ether. A portion of this extract is cleaned up with a solid-phase extraction aminopropyl disposable column. With LC analysis using an RP-8-DB microbore column, acetonitrile–water (70 + 30, v/v) as mobile phase, and photodiode array detection at 254 nm, recovery and repeatability data were collected for the 5 benzoylureas on 4 vegetables and citrus in the range 0.04–2.0 mg/kg. Validated limits of detection and quantitation were 0.01 and 0.04 mg/kg, respectively. The method is reliable for routine analysis of vegetables and fruits.

Benzoylureas are a group of insecticides that inhibit chitin synthesis and thus interfere with formation of the cuticle and further growth of the insect. The compounds lack plant systemic action and thus do not penetrate plant tissue.

Gas chromatography (GC) with electron capture or mass spectrometric (MS) detection has been used to analyze benzoylureas after derivatization with heptafluorobutyric anhydride to avoid thermal degradation (1–4). Reversed-phase liquid chromatograph (LC) with UV detection is also widely used for analysis of plant material (5–7), animal tissues (8, 9), and forestry substracts (10) that are thermally unstable. LC/MS has been used to analyze these compounds in crops (11, 12).

We report a rapid and sensitive LC method for determining 5 benzoylureas—diflubenzuron, hexaflumuron, teflubenzuron, flufenoxuron, and lufenuron—in plant material.

The most common cleanup technique for extracts containing benzoylureas residues are liquid–liquid partitioning, adsorption chromatography, and gel permeation chromatography. However, solid-phase extraction (SPE) can reduce analysis time and costs.

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Our goal was to perform a simple, rapid, and sensitive routine LC method with a portion of an extract obtained for multiresidue and GC methods (13) and for analysis of *N*-methylcarbamates (14) and benzimidazoles (15).

Experimental

Chemicals

(**a**) *Standard materials.*—All standard materials were of the highest purity available. Diflubenzuron was 99.5% pure from Solvay Duphar (Weesp, The Netherlands), hexaflumuron was 99.6% pure from Dow-Elanco (Middlesex, UK), teflubenzuron was 99.5% pure from Cyanamid (Princeton, NJ), lufenuron was 99.7% pure from Ciba Geigy (Basel, Switzerland), and flufenoxuron was 96.1% pure from Shell (Sittingbourne, Kent, UK). For each standard, a stock solution of 1 mg/mL in acetonitrile was prepared and stored at 6°C. Working standards solutions (0.1–1.0 mg/L) were prepared in the mobile phase.

(**b**) *Spike standard stock solution.*—One hundred milligrams of each benzoylurea standard was dissolved in 100 mL dichloromethane to obtain a 1 mg/mL stock solution. Dilute standard mixture was prepared by transferring 100 µL of each standard in a volumetric flash and diluting to 100 mL with dichloromethane to obtain 1 µg/mL standard mixture of 5 benzoylureas.

(**c**) *Solvents.*—Acetone, dichloromethane, *n*-hexane, methanol, petroleum ether (b.p., 4°–60°C) were all pesticide residue quality from Scharlau (Barcelona, Spain). Acetonitrile and dioxane were LC grade from Scharlau, and water was LC grade, obtained from a Nanopure II system (Warnstead, Dubuque, IA). MTBE (methyl *tert*-ether) was from Merck (Darmstad, Germany).

(**d**) *SPE cartridges.*—6 cc/1 g, Mega Bond Elut, aminopropyl bonded-phase columns (Varian/Analytichem, Code 0319; Harbor City, CA).

(**e**) *Filters.*—Millex LCR13 LC certified 0.5 µm, No. SLCR013NS (Millipore, Bedford, MA).

Instruments

(**a**) *Homogenizer.*—Heidolph Diax 600 (Schawabach, Germany).

(**b**) *Centrifuge.*—Hereaus Sepatech Model Labofuge GL (Hanau, Germany).

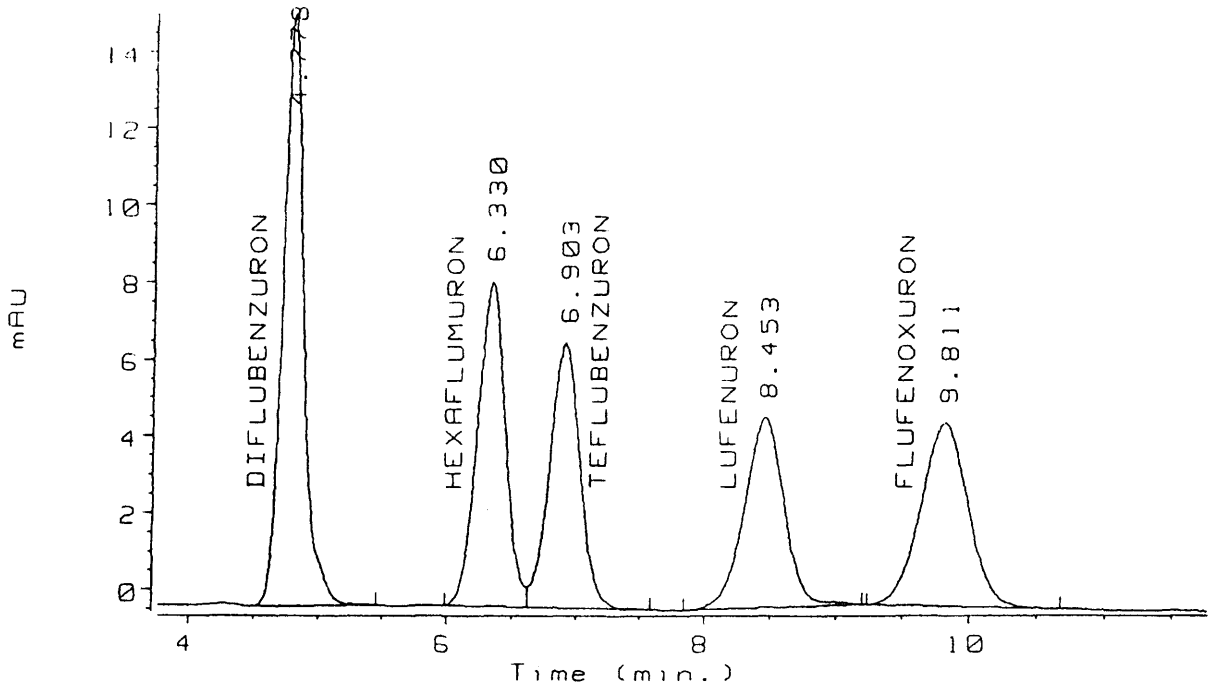

Figure 1. Chromatogram of standard mixture containing 15 ng of each benzoylurea on-column. Mobile phase, CH_3CN–H_2O (70 + 30, v/v); flow rate, 0.25 mL/min; photodiode array detection at 254 nm.

(**c**) *Food chopper.*—Dito-Sama K-55 (Aubusson, France).

(**d**) *LC system.*—Model 1090 Series II liquid chromatograph (Hewlett-Packard, Palo Alto, CA) with a ternary gradient feature, an autoinjection system, and a photodiode array detector. The instrument was controlled with a Model 9000 Series ChemStation using LC-Pascal software (Rev. 5.22). Benzoylureas were separated with a Supercosil LC-8-DB column (5 μm, 25 cm × 2.1 mm id) from Supelco (Bellefonte, PA) and a guard column cartridge ODS Hypersyl (5 μm, 20 × 2.1 mm id) from Hewlett-Packard. Data were acquired at 254 nm with a 20 nm bandwidth. The reference signal was at 450 nm with a 100 nm bandwidth. Detector sensitivity was set at 10 milliabsorbance units full scale. Separation was performed under isocratic conditions at room temperature. The injection volume was 15 μL, and the flow rate was 0.25 mL/min (Figure 1). The mobile phase was CH_3CN–H_2O (70 + 30, v/v) for all benzoylureas in every matrix tested, except for diflubenzuron in oranges, in which case CH_3CN–H_2O–dioxane (45 + 45 + 10, v/v/v) was used.

Analytical Procedure

(**a**) *Extraction.*—A representative sample of whole fruit or vegetable (15 g) was weighed into a 250 mL Teflon centrifuge bottle and then homogenized with 30 mL acetone for 30 s. Sixty milliliters dichloromethane–petroleum ether (50 + 50) was added, and the mixture was homogenized for 1 min. After centrifugation of homogenate for 5 min at 4000 rpm, the upper layer (organic phase) was decanted into a graduated flask, and the volume of extract was measured (usually ca 85 mL). Twenty-five milliliters of the extract was concentrated to dryness in a rotary evaporator with a bath water at 35°C. The residue was dissolved in 2 mL *n*-hexane.

(**b**) *SPE cleanup.*—A Mega Bond Elut cartridge was washed with 15 mL *n*-hexane. The sample, dissolved in 2 mL *n*-hexane, was applied to the cartridge, and then the cartridge was rinsed with 9 mL *n*-hexane and with 8 mL MTBE–*n*-hexane (20 + 80, v/v). The eluates were discarded. Analytes were eluted with 5 mL acetone, and the eluate was evaporated to dryness in a rotary evaporator. The dry residue was dissolved in 0.5 mL acetonitrile, and 0.5 mL water was added. This extract was filtered over a 0.5 μm filter, and the filtrate was collected in a 2 mL autosampler vial.

Results and Discussion

This method for benzoylureas is fast and inexpensive, and it can be used for routine analysis of vegetables and citrus fruits. It also allows use of extract prepared for multiresidue methods (16–18) or for analysis of other analytes, such as benzimidazoles and *N*-methylcarbamates. The procedure does not involve partitioning and laborious cleanup. SPE aminopropyl disposable columns are used to eliminate interferences.

The method gives good recoveries. Matrix interferences in vegetables are reduced to a minimum and are well separated from the analytes through the optimized isocratic run with UV detection at 254 nm (Figures 2 and 3).

Citrus samples included peel and pulp and interferences such as essential oils and waxes from the peel interfered with

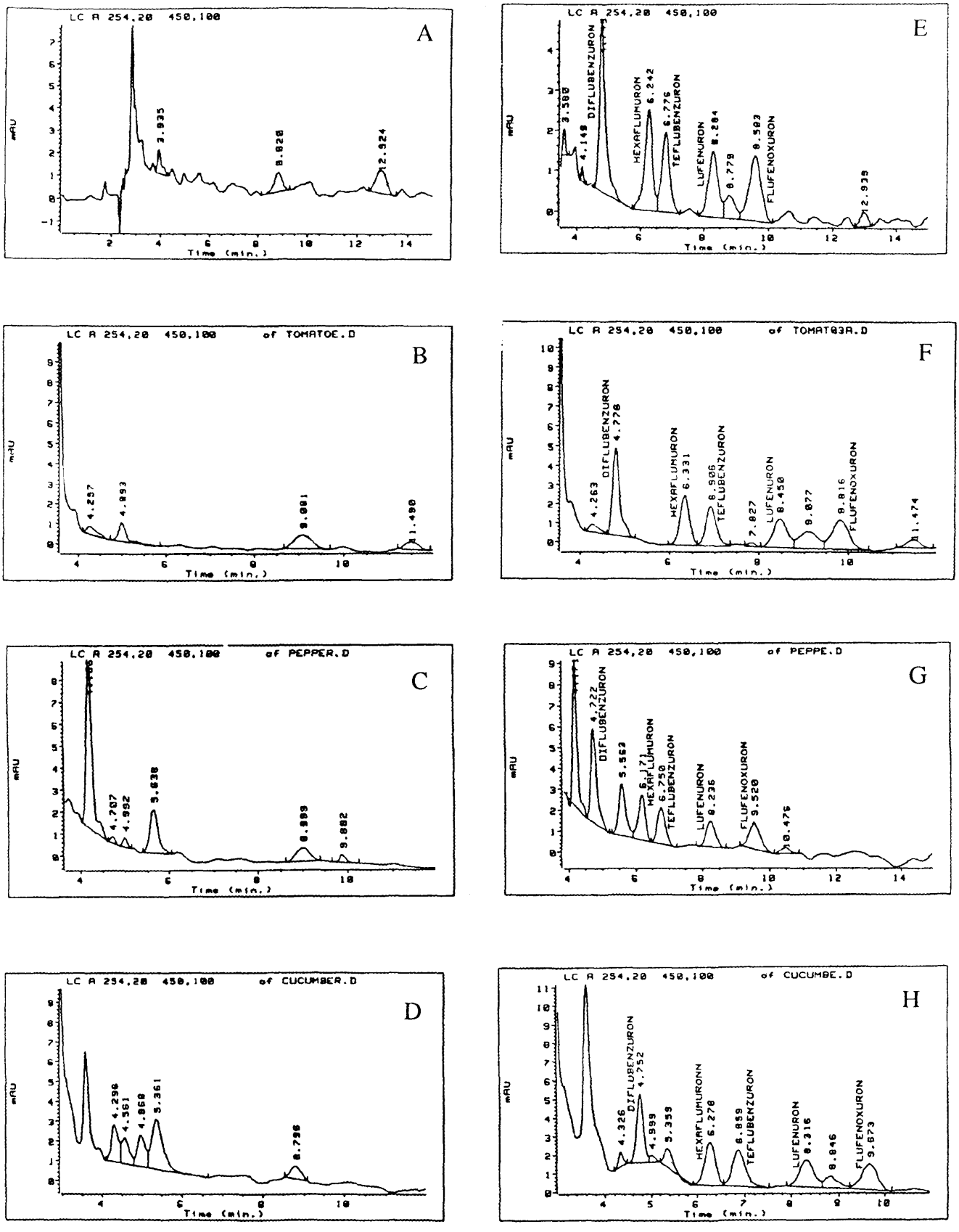

Figure 2. Chromatograms of untreated extract—(A) eggplants, (B) tomatoes, (C) peppers, and (D) cucumbers—and samples spiked with benzoylureas at 0.13 mg/kg—(E) eggplants, (F) tomatoes, (G) peppers, and (H) cucumbers. Same conditions as described in Figure 1.

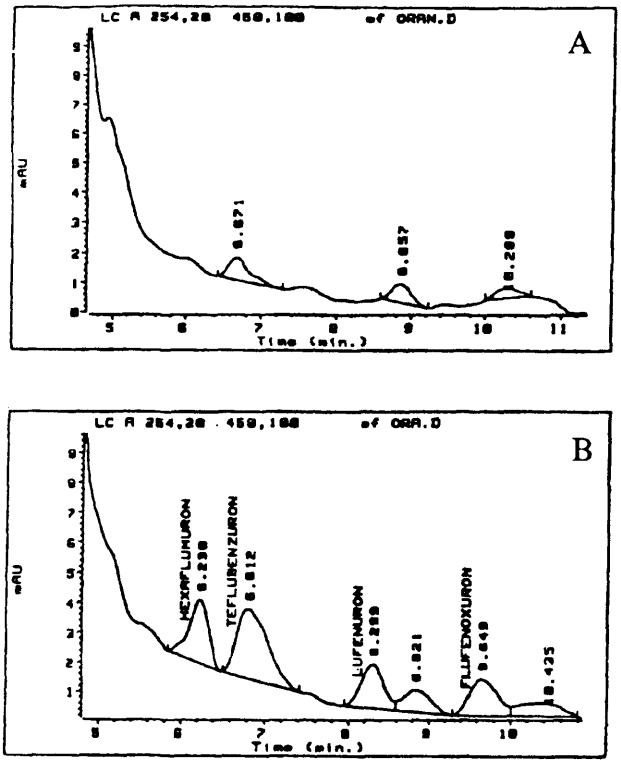

Figure 3. Chromatograms of (A) untreated oranges and (B) oranges spiked with benzoylureas at 0.13 mg/kg. Same conditions as described in Figure 1.

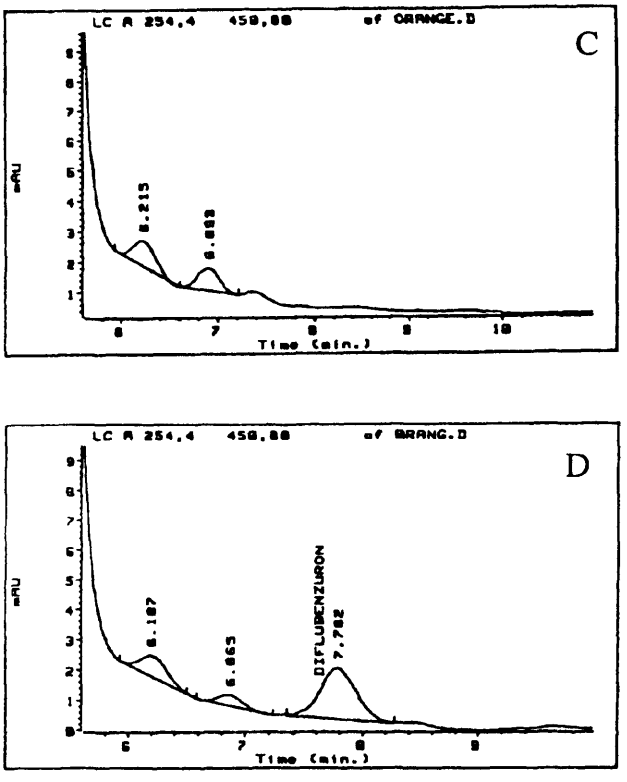

Figure 5. Chromatograms of (C) orange extract after cleanup and (D) sample spiked with diflubenzuron at 0.13 mg/kg. Same conditions as described in Figure 4.

determination of diflubenzuron. This problem was solved by modifying the mobile phase with dioxane while using the same analytical conditions (Figures 4 and 5).

Recoveries from homogenized untreated peppers, tomatoes, cucumbers, eggplants, and oranges spiked with each analyte at 0.04, 0.13, and 2.0 mg/kg were good (Table 1). Mean recoveries were between 58 and 118%, with relative standard deviation (RSD) values between 2 and 22%. These are considered acceptable for these types of matrix.

In some cases, higher concentrations (e.g., 2.0 mg/kg) of analytes overloaded the solid-phase column, giving lower recoveries compared with other levels. For example, mean recov-

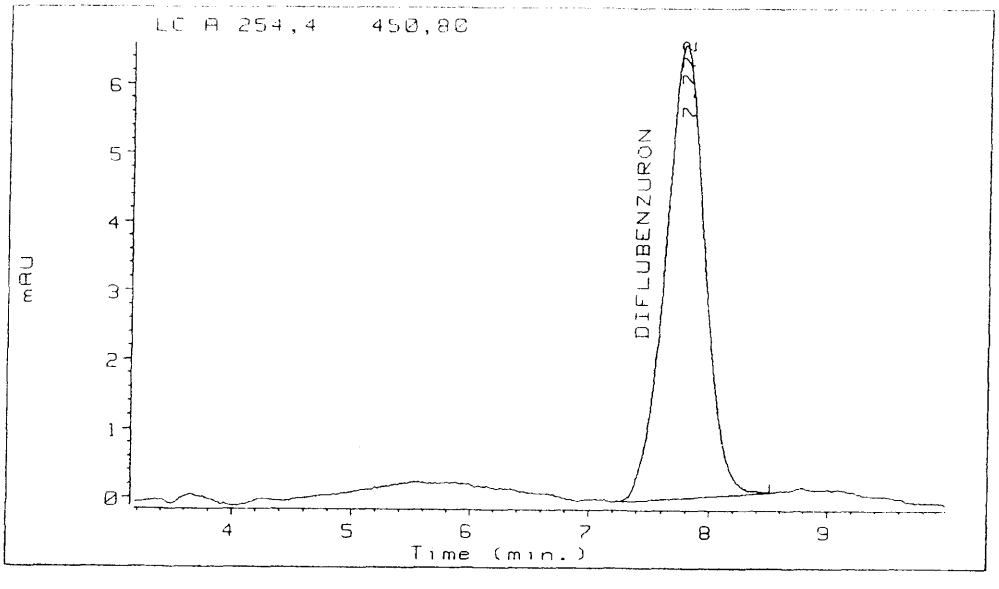

Figure 4. Chromatogram of diflubenzuron standard containing 15 ng diflubenzuron on-column. Mobile phase, CH_3CN–H_2O–dioxane (45 + 45 + 10, v/v/v); flow rate, 0.25 mL/min; photodiode array detection at 254 nm.

Table 1. Recovery of 5 benzoylureas from spiked peppers, tomatoes, cucumbers, eggplants, and oranges

		Diflubenzuron				Hexaflumuron				Teflubenzuron				Lufenuron				Flufenoxuron			
Matrix	Spike, mg/kg	Range, %	*n*	Mean, %	RSD, %	Range, %	*n*	Mean, %	RSD, %	Range, %	*n*	Mean, %	RSD, %	Range, %	*n*	Mean, %	RSD, %	Range, %	*n*	Mean, %	RSD, %
Peppers	2.0	67–86	6	72.8	9.5	59–74	6	61.9	9.7	56–71	6	60.5	8.7	55–69	6	59.6	8.2	54–66	6	57.8	7.6
	0.13	81–94	7	84.4	4.7	77–90	7	79.3	6.2	74–87	6	79.6	5.2	65–103	7	82	13.8	57–100	7	85.6	14.5
	0.04	84–133	6	110	14.2	66–80	6	72.9	6.6	64–73	6	70.1	5.6	55–79	6	68.5	13.1	81–124	6	110	15.4
Tomatoes	2.0	67–97	6	81.5	14.8	65–95	6	79.4	14.8	64–95	6	78.8	15.2	63–94	6	77.5	15.4	62–93	6	76.8	15.3
	0.13	73–102	7	88.7	9.8	88–100	7	94.8	4.4	95–100	6	98.1	2.1	98–117	7	95.8	9.7	87–110	7	95.8	9.4
	0.04	76–89	6	83.1	6.7	82–93	6	87.2	5.2	66–87	6	77.5	9.9	83–98	6	90.2	7.3	91–123	6	108	9.9
Cucumbers	2.0	75–84	6	79.7	4.4	72–82	6	77.4	4.9	72–85	6	79.2	6.2	69–85	6	79.5	8.3	63–79	6	72.2	7.5
	0.13	57–74	7	65.1	6.8	70–106	7	88.5	13.8	80–102	6	90.3	9.9	67–97	7	82.2	10.9	63–94	7	83.2	10.9
	0.04	75–87	6	80.4	6.5	73–97	6	85.2	9.8	93–117	6	105	9.6	78–100	6	89.6	9.9	91–108	6	98.8	6.7
Eggplants	2.0	64–98	6	83.5	16.5	59–91	6	76.5	16.8	47–92	6	74.7	22.7	58–95	6	77.2	18.6	58–90	6	74.8	16.6
	0.13	64–105	7	87.4	15.6	77–109	7	94.4	11.5	70–117	6	99.8	16	77–130	7	99	22.1	89–106	7	96.1	6.1
	0.04	80–112	6	101	11.4	54–74	6	67.1	10.4	96–138	6	118	12.2	—[a]	—	—	—	77–94	6	84.3	10.5
Oranges	2.0	67–84	6	74.9	9.1	54–72	6	63.4	9.6	53–71	6	63.2	9.9	65–86	6	77.1	10.4	56–76	6	67.2	10.2
	0.13	56–80	7	69.2	7.5	67–90	7	78.4	8.8	75–94	6	83.1	7.9	84–109	7	93.5	11.1	81–97	7	86.7	5.9
	0.04	61–100	6	73.5	19	90–108	6	98.4	8.2	73–99	6	86.8	11.6	64–82	6	71.9	8.8	87–104	6	97.5	6.4

[a] —, matrix interferences appear at retention time of lufenuron (0.04 mg/kg).

ery of teflubenzuron in oranges spiked at 2.0 mg/kg was 63%, compared with 83 and 87% for oranges spiked at 0.13 and 0.04 mg/kg, respectively.

The estimated limit of detection for benzoylureas in the tested matrixes was 0.01 mg/kg, and the limit of determination was 0.04 mg/kg, except for lufenuron in eggplants (0.04 and 0.10 mg/kg, respectively) because of matrix interferences.

Conclusions

A rapid and simple procedure for determining benzoylureas in crops and fruits was developed. Recovery and repeatability values are good for these kind of matrixes. For samples with high essential oil and wax contents, such as whole citrus fruit, a slight modification of the mobile phase composition is required to determine diflubenzuron. An additional advantage of this method is that an extract such as the one commonly obtained in the Luke procedure can be used, facilitating application of the method for routine purposes.

Acknowledgment

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