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Short communication

Determination of benzoylurea insecticides in apples by high-performance liquid chromatography

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Abstract

A method for the simultaneous determination of the benzoylurea insecticides diflubenzuron, flufenoxuron, flucycloxuron chlorfluazuron and triflumuron in apples using reversed-phase HPLC is described. The separation of analytes was performed on a Separon SGX C_8 column (150 × 3 mm I.D., 5 μm) with methanol–water (55:45, v/v) as the mobile phase. Benzoylurea residues were extracted with acetone and then partitioned into dichloromethane. The final clean-up step was conducted by gel permeation chromatography on Bio- Beads SX-3 using cyclohexane–chloroform (3:2, v/v) as the mobile phase.

1. Introduction

Benzoylureas (BU) are promising insecticides, used for the control of insects attacking a wide range of crops, especially fruits and vegetables. These compounds act as powerful growth regulators which inhibit the synthesis of cuticle chitin in target pests, causing death or abortive development [1–3]. BUs are considered to be a fourth generation of insecticides with many attractive properties such as high selectivity, low acute toxicity for mammals and high biological activity, resulting in low application rates [4,5].

There are nine BUs registered [6] in the Czech Republic as active ingredients of pesticide formulations (apples being the main protected crop). Structures and solubility data for the most common BUs are shown in Table 1.

Reversed-phase HPLC with UV detection is predominantly employed for the determination of BUs [7–12] because they are thermally unstable and, consequently, their GC properties are poor [7,13]. The latter technique has occasionally been applied after derivatization of the parent analytes or their hydrolytic products [7,14,15].

Most of the above analytical studies were concerned with the determination of a single BU only. In this paper, a rapid isocratic reversed-phase HPLC method applicable to the simultaneous determination of five extensively used BUs is described. Whereas time-consuming multiple partitioning of extracts followed by adsorption chromatography on Florisil was commonly utilized in previous methods, in this work

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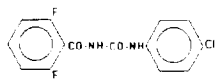
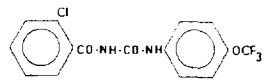
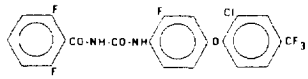
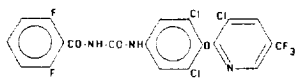
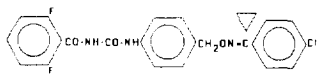
Table 1
Structures and solubility data for the most common BUs

Common name	Structure	Molecular mass	Solubility in water (mg/l)
Diflubenzuron		310	0.1
Triflumuron		358	<0.001
Flufenoxuron		488	0.001
Chlorfluazuron		540	<0.01
Flucycloxuron		483	<0.001

a significant simplification of the clean-up step was achieved by using gel permeation chromatography (GPC).

2. Experimental

2.1. Materials

All chemicals were of analytical-reagent grade. Methanol, cyclohexane, acetonitrile and chloroform (Lachema, Brno, Czech Republic) were glass distilled prior to the use. Anhydrous sodium sulphate was purified by heating at 600°C for 3 h. Standards of BUs (diflubenzuron, flucycloxuron, flufenoxuron, triflumuron and chlorfluazuron) of purity not less than 99% were provided by Solvay-Duphar, Shell International Petroleum, Bayer and Ciba-Geigy. Stock standard solutions prepared by dissolution in methanol were stable for 2 weeks when held at 4°C.

2.2. Equipment

A Hewlett-Packard Model 1090 M liquid chromatograph equipped with an HP Model 1040 M diode-array detector was used. The columns were CGC glass cartridges (150 × 3 mm I.D.) filled with (i) Separon SGX C_{18}, (ii) Separon SGX Phenyl and (iii) Separon SGX C_8, all 5 μm (Tessek, Prague, Czech Republic). Separation of BUs was tested on these columns at 40°C using methanol–water and acetonitrile–water mixtures as mobile phases at a flow-rate of 0.5 ml/min. The detection wavelength was set at 260 nm.

Gel permeation chromatography was performed on a 500 × 8 mm I.D. column filled with Bio Beads SX-3, 200–400 mesh (Bio-Rad, Richmond, CA, USA). Mobile phases consisting of cyclohexane–chloroform (1:1, 3:2 and 4:1, v/v) were tested. The flow-rate was 0.6 ml/min and a 2-ml sample loop was used.

2.3. Sample preparation

GPC: determination of the elution profile

Volumes of 2 ml of standard solutions of BUs or crude apple extract (see below), dissolved in the mobile phase, were loaded on to the column. Fifteen 2-ml fractions of the eluate were collected in order to obtain an elution curve. The amount of apple wax and other co-extracts in each fraction was determined gravimetricaly

after evaporation of solvent. BUs were determined by HPLC after dissolving residue in the mobile phase.

Isolation of BUs from apples

A 50-g amount of a representative sample was homogenized in 100 ml of acetone. The homogenate was filtered through glass-wool into a separating funnel. After addition of 10 g of NaCl and 10 ml of distilled water, partitioning with 50 ml of dichloromethane was carried out. The organic layer was separated and dried over anhydrous sodium sulphate. After evaporation to dryness, the residue was dissolved prior to GPC clean-up in the respective mobile phase [cyclohexane–chloroform (3:2, v/v)].

Isolation of BUs from surface layer of apples

Approximately 200 g of apples (intact fruit) were washed by immersing them in two 150-ml portions of dichloromethane in an appropriately shaped beaker. The combined extracts were dried over anhydrous sodium sulphate and then evaporated to dryness. After dissolving of residue in cyclohexane–chloroform, an aliquot corresponding to 50 g of apple was cleaned up by GPC.

3. Results and discussion

Despite the effort spent on optimization of the HPLC conditions, it was not possible to achieve a satisfactory resolution of all analytes on an HPLC column packed with reversed-phase C_{18}: flucycloxuron and flufenoxuron exhibited very similar k' values in tested mobile phases [16]. For this reason, the separation of BUs on several other stationary phases was examined. The best results were achieved on the Separon SGX C_8 column. All analytes were resolved under isocratic conditions with methanol–water (55:45, v/v) as the mobile phase. The time of analysis did not exceed 10 min. Detection at 260 nm provided the most sensitive and relatively selective detection of the analytes. Minimum detectable amounts are summarized in Table 2. The values recorded for diflubenzuron and flufenoxuron are comparable to those published by other workers [8,12]; there are no previous data available for the remaining BUs.

"Classical" clean-up based on adsorption chromatography was replaced by GPC in this work. Mobile phases consisting of cyclohexane–chloroform in three different proportions (4:1, 3:2 and 1:1, v/v), i.e., mixtures with different "polarity", were tested in preliminary experiments. An increased content of chloroform in the mobile phase resulted in narrower elution bands of co-extracts (see Fig. 1). No significant differences in the elution patterns of compounds extracted from homogenized (disintegrated) apples and those washed from the surface of the intact fruit were observed. The best resolution of BUs from co-extracts was obtained using the GPC system employing cyclohexane–chloroform (2:3, v/v) as the mobile phase. The main portion (approximately 95%) of co-extracts was eluted in a volume of 6–14 ml and, as can be seen from Fig. 2, elution of BUs occurred afterwards, in the

Table 2
Detection limits of BUs with relative standard deviations

Compound	Minimum detectable amount (ng)	Method detection limit (mg/kg)	R.S.D. (%)[a]	
			0.1 mg/kg level	1 mg/kg level
Diflubenzuron	5	0.01	9	5
Flufenoxuron	8	0.02	9	7
Flucycloxuron	10	0.02	13	10
Chlorfluazuron	15	0.03	11	9
Triflumuron	5	0.01	14	9

[a] $n = 4$.

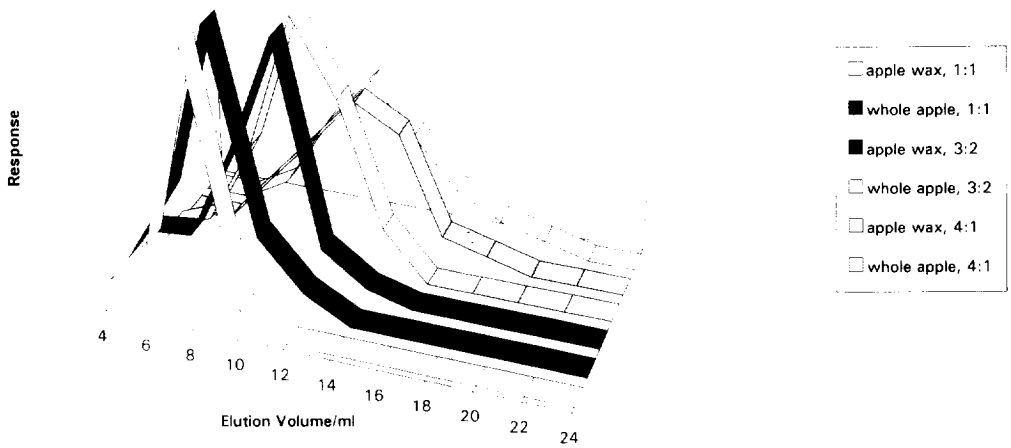


Fig. 1. Elution profiles of co-extracts from (whole) apples and apple wax in three mobile phases with different cyclohexane–chloroform ratios.

range 14–22 ml. A size-exclusion mechanism evidently predominated in the GPC system employed. In accordance with theory, BUs were eluted in order of their molecular mass (Table 1), i.e., in the descending order chlorfluazuron, flufenoxuron, flucycloxuron, trifumuron and diflubenzuron.

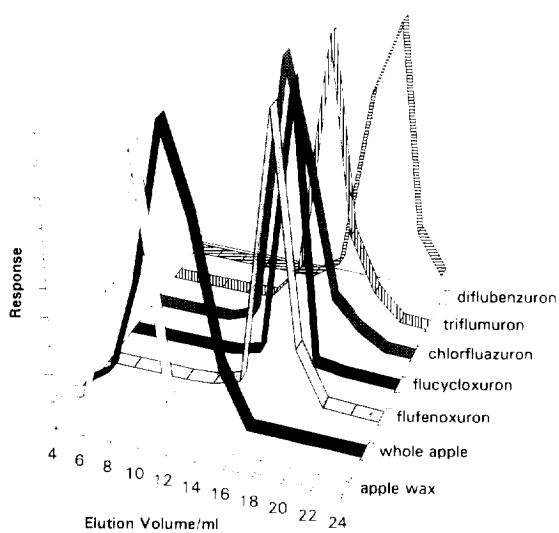


Fig. 2. Elution profiles of BUs and co-extracts from (whole) apples and apple wax. Mobile phase: cyclohexane–chloroform (3:2, v/v).

The efficiency of the applied clean-up procedure for isolated surface wax (which can be assumed to represent the main portion of co-extracts) is well illustrated in Fig. 3, showing chromatograms of apple extracts (blank and spiked) together with a standard mixture. Several additional co-extracts adsorbing at 260 nm appeared in chromatograms corresponding to extracts obtained from homogenized apples (Fig. 3c), but no interference with the peaks of the analytes occurred. The recoveries of diflubenzuron, flufenoxuron, flucycloxuron, chlorfluazuron and triflumuron measured at the 0.1 mg/kg level were 92, 72, 75, 71 and 79%, respectively.

4. Conclusions

A reversed-phase (C_8-bonded silica) HPLC method employing GPC clean-up (Bio Beads SX-3) can be recommended for the sensitive and

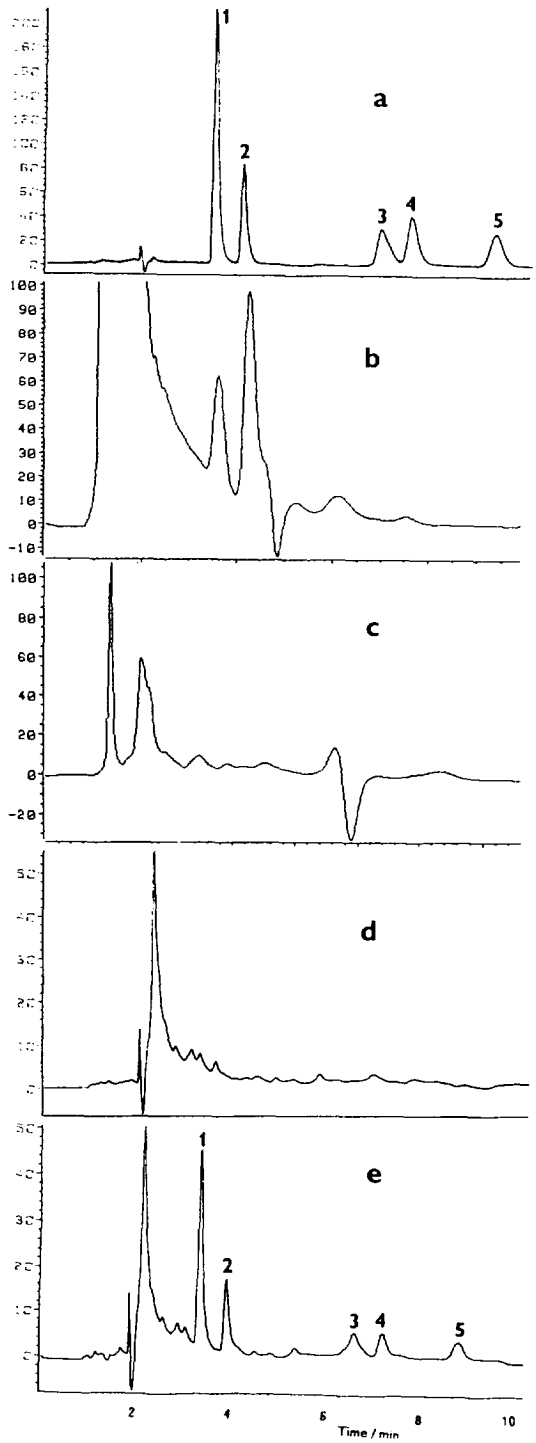


Fig. 3. Chromatograms of (a) standard mixture of BUs (1 diflubenzuron; 2 triflumuron; 3 flufycloxuron; 4 flufenoxuron; 5 chlorfluazuron), (b) crude apple extract, (c) apple extract after clean-up, (d) apple wax after clean-up and (e) apple wax spiked at 0.05 mg/kg with flucycloxuron, flufenoxuron and chlorfluazuron and 0.1 mg/kg with diflubenzuron and triflumuron.

reproducible determination of BU residues in apples. Because of their hydrophobic nature, BU residues are expected to be located predominantly in the surface layers of treated fruit, dissolved in the wax layer, As a result, homogenization of samples and more complicated isolation of residues from homogenates can be avoided by a simpler and more rapid examination (especially for screening purposes) of wax contamination.

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References

[1] N.P. Hajjar and J.E. Casida, Science, 200 (1978) 1499.
[2] A.R. Horowitz, M. Klein, S. Yablonski and I. Ishaaya, Crop Protect., 11 (1992) 465.
[3] R. Mussarelli, Chitin in Nature and Technology, Plenum Press, New York, 1986.
[4] R.L. Metcalf, P. Lu and S. Bowlus, J. Agric. Food Chem., 23 (1975) 359.
[5] C.R. Worthing, The Pesticide Manual, British Crop Protection Council, 9th ed., 1990.
[6] List of Approved Agents for Plant Preservation, Czech Ministry of Agriculture, 1994.
[7] C. Corley, R.W. Miller and K.R. Hill, J. Assoc. Off. Anal. Chem., 57 (1974) 1269.
[8] E.R. Bogus, P.A. Gallagher, E.A. Cameron and R.O. Mumma, J. Agric. Food Chem., 33 (1985) 1018.
[9] D.J. Austin and K.J. Hall, J. Pestic. Sci., 12 (1981) 495.
[10] D.J. Austin and K.J. Carter, J. Pestic. Sci., 17 (1986) 73.
[11] W.A. Hopkins and D.R. Lauren, J. Chromatogr., 516 (1990) 442.
[12] K.M.S. Sundram and R. Nott, J. Liq. Chromatogr., 12 (1989) 2333.
[13] S. Smith, G.H. Willis and L.L. McDowell, J. Agric. Food Chem., 31 (1983) 610.
[14] N.H. Nigg, R.D. Canizzaro and J.H. Stamper, Bull. Environ. Contam. Toxicol., 36 (1986) 833.
[15] H.J. Stan and P. Klaffenbach, Fresenius' J. Anal. Chem., 339 (1991) 40.
[16] T. Tomšej, J. Hajšlová, P. Outrata and J. Landesková, Agrochémia, 33 (1993) 255.