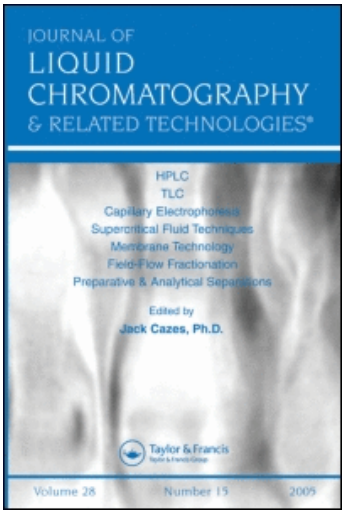

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Determination of 4-Chloroaniline and 4-Chlorophehyl Urea in Honey By Liquid Chromatography with Electrochemical Detection

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DETERMINATION OF 4-CHLOROANILINE AND 4-CHLOROPHEHYL UREA IN HONEY BY LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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ABSTRACT

A liquid chromatographic technique with amperometric detection was developed for determining diflubenzuron metabolites, 4-chlorophenyl urea and 4-chloroaniline, using a Spherisorb C_{18} column 5 μm (4.1 x 250 mm). A mobile phase of acetonitrile / citrate-disodium hydrogenphosphate buffer, 30:70, at pH=7.0, was pumped at a flow rate of 1 mL min^{-1}. The amperometric detector, equipped with a glassy carbon working electrode was operated at 1100 mV vs Ag/AgCl. The method showed a limit of detection of 0.46 μg l^{-1} for 4-chlorophenyl urea and 0.20 μg l^{-1} for 4-chloroaniline. Reproducibility in terms of relative standard deviation ranged between 1.24 and 4.50 % for the 4-chlorophenyl urea and 1.60 and 3.93% for the 4-chloroaniline. The method was applied to the determination of both metabolites in honey. The prior extraction of the two substances from honey was established using different solid-liquid extraction phases.

INTRODUCTION

Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea)], is a chemical used to control numerous forest and agricultural pests.[1-3] It acts through disruption of the synthesis of new cuticle at moults and is, therefore, arthropod specific.

Generally it only has short-term effects against sensitive nontargets and it is not persistent in habitats where it would be applied. However, it is degraded quickly in the environment to the metabolites 4-chlorophenyl urea and 4-chloroaniline. These compounds are classified as mutagens by the National Cancer Institute and the Cancer Assessment Group of the U. S. Environmental Protection Agency.

The environmental fate of diflubenzuron has been studied in the laboratory and in field.[4-7] Several of these studies have shown that diflubenzuron normally persists for only about 2 to 3 days. However, persistence is related to water quality and climatic conditions, and low concentrations can persist for 7 weeks or longer in pond waters under low pH and low temperatures. The major degradation products of diflubenzuron are 2,6-diflurobenzamide, 2,6-diflurobenzoic acid, 4-chlorophenyl urea and 4-chloroaniline.

Determination of phenylurea herbicides and their metabolites has been mainly performed by gas chromatography (GC) and liquid chromatography (LC). Some phenylureas and anilines cannot be analyzed by GC because of their thermolability and polar character.[8] In these cases the derivatization before injection has been used.[9-11] Bromination[12] or acylation with heptafluorobutyric anhydride,[13] both of which enable sensitive determination by electron capture detection (ECD), have been used in GC separation. More recently, attempts have been made to analyze substituted anilines by direct GC without derivatization: Boër et al.[14] used cold column injection for analysis of thirteen substituted anilines in environmental and drinking waters by direct capillary GC with nitrogen-phosphorus detection (NPD) on a DB-Wax column. Phenylurea herbicides interfered with the determination when present at relatively high levels.

For such reasons HPLC is the more frequently used method. Most authors[15-18] have employed reversed phase chromatography and UV detection but, better sensitivity and selectivity can be achieved with the electrochemical detector (ED).

Hatrik[19] used tandem UV and ED for the simultaneous determination of phenylurea herbicides which have been widely used in agriculture and for the determination of their corresponding degradation products in water samples.

Due to aerial treatments used to apply the diflubenzuron in big forest areas, some close apiaries could be affected. Studies with other pesticides like malathion and dimethoate have been made.[20] For this reason, quality control of the honey is necessary. Most authors use GC with different detection modes for determination of pesticides in honey.[21,22] However, some have used HPLC-UV for determination of these compounds. Thus, cymazole and residues were determined with limit of determination of 0.01 mg l^{-1}.[23]

However, the presence of diflubenzuron major metabolite, 4-chlorophenyl urea, was detected sporadically at minor levels.[24] In this work, electrochemical detection has been used for the simultaneous determination of this substance and its degradation product, 4-chloroaniline. Diflubenzuron and metabolites determination methods in honey were not found, even though different toxicology studies have been carried out.[25]

MATERIALS

Apparatus

The instruments used for the cyclic voltammetry studies were a Metrohm (Metrohm Ltd. Herisau, SW) E-506 Polarecord, a Model E-612 scanner, a VA-663 unit stand and an Yokogawa (Yokogawa Europe, BV, Amersfoort ND) X-Y recorder. A glassy carbon (GC) Metrohm was used as the working electrode, a platinum electrode as the auxiliary, and an Ag/AgCl electrode as the reference.

The isocratic HPLC system used was a Hewlett Packard (Palo Alto, CA, USA) series 1050 pump, and a Waters (Waters Assoc. Milford M.A. USA) model 460 amperometric detector with a 2.5 μL microcell, a GC model 41215 electrode and a Ag/AgCl reference electrode. The chromatograms were recorded using a Waters Model 745 data module.

The column was a 25.0 cm x 4.0 mm, I.D. 5 mm Spherisorb C18 (Tracer Anal. Barcelona SP). A centrifuge Selecta (Barcelona SP) Model Centronic and a Selecta Model Vibromatic-384 were used in the extraction procedure.

A Vac-Elut sample preconcentration system (Varian, Harbor City CA. USA) for solid-phase extraction was used in the purification procedure. In the solid phase extraction three types of Sep-Pack cartridges (Waters Associates, Milford, MA., USA) were used: florisil, cyanopropyl and aminopropyl containing 500 mg of sorbent.

Chemicals and Solvents

The standards 4-chlorophenyl urea and 4-chloroaniline were supplied by the Laboratory of Dr. Ehrenstorfer GmbH (Angsburg, Germany) with a purity of 99.0 and 99.7% respectively. The internal standard todralazine was obtained from Sigma (St. Louis, MO, USA)

Standard stock solutions were prepared by dissolution of 10 mg of 4-chloroaniline and 4-chlorophenyl urea in 100 mL of acetonitrile.

All chemicals were of analytical-reagent grade. The solvents used in the mobile phase were of HPLC-grade and were obtained from BDH (Poole, Dorser, UK). Helium was of oxygen-free grade (Air Liquide, Madrid, SP).

HPLC-grade water was prepared by purifying demineralized water in a Milli-Q filtration system (Millipore, Bedford, MA, USA). All solvents and samples were filtered through 0.22 μm Millipore membrane filters before injection.

METHODS

Chromatographic Conditions

The supporting electrolyte used in the mobile phase was prepared by mixing $5.0x10^{-3}$ mol l^{-1} citric acid and $1.0x10^{-2}$ mol l^{-1} disodium hydrogen phosphate to yield the desired pH. The resulting buffer solution was diluted with acetonitrile to obtain the desired concentrations.

The mobil phase was sonicated for 15 min and the eluent reservoir was purged with helium before use. The column head-pressure was 1732 psi at a flow rate of 1.0 mL min^{-1}. The system was operated at room temperature and injection volume was 20 μL.

Preparation of Spiked Samples

For recovery determinations, samples of honey, 10 g, were spiked by the addition of an appropriate amount of a standard stock solution (1 μg mL^{-1} in acetonitrile). The spiked samples were allowed to stand for a few minutes before extraction to allow the spike solution to penetrate the sample. The efficiency of the method was determined by fortifying control samples over a concentration of 10 to 60 mg Kg^{-1}.

Extraction and Clean-up

A 10 g sample of honey heated to 30°C was extracted in a capped centrifuge tube with 5 mL of acetonitrile shaken for 10 min. This mixture was centrifuged at 3000 rpm (1000 g) for 5 min and filtered by suction.

The three different solid-liquid extraction phases (aminopropyl, cyanopropyl and fluorisil) were washed with 10 mL of acetonitrile for activation of the sorbent. The sample extract was passed through the cartridge by negative pressure of 7 in of Hg with a Vac-Elut station.

A 200 μL of 1 μg mL^{-1} solution of internal standard todralazine was added to 1.0 mL of the liquid that passes through the cartridge and contains 4-chlorophenyl urea and 4-chloroaniline, and then aliquots of 20 μL were injected into the HPLC system.

RESULTS AND DISCUSSION

The presence of oxidizable amine groups on the glassy carbon electrode can be used for electroanalytical determination of 4-chloroaniline, 4-chlorophenyl urea and todralazine

Figure 1 shows the current-potential curves obtained for these substances with cyclic voltammetry. On the anodic scan at pH=7.0, the compounds exhibited broad irreversible oxidation waves at different potentials: 570, 438 and 462 mV, respectively. This showed that the three substances can be jointly determined by HPLC-ED chosing an appropriate working potential that assures the oxidation of all the groups.

Hydrodynamic voltammograms were used to determine the optimum pH, and potential conditions for the electrochemical detection. The graphs were derived by injecting 20 ng of 4-chlorophenil urea, 10 ng of 4-chloroaniline and

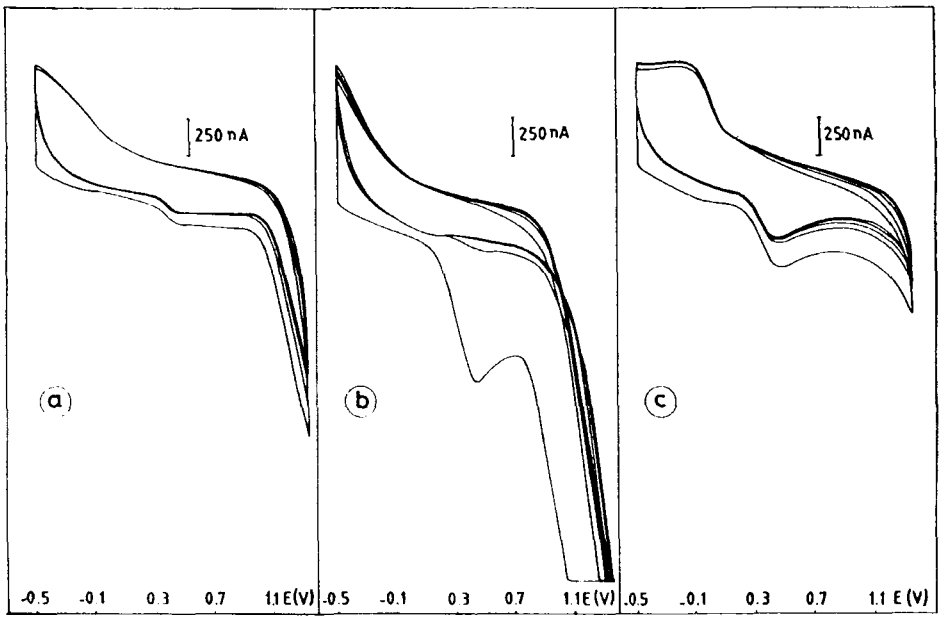

Figure 1. Cyclic voltammograms of (a) 4-chlorophenyl urea, (b) 4-chloroaniline, (c) todralazine, on glassy carbon electrode. Concentration: 1.0×10^{-4} mol l^{-1}. Scan rate: 400 mV s^{-1}. Solvent: Acetonitrile / citric acid - disodium hydrogenphosphate buffer (30:70), pH=7.0

20 ng of todralazine and varying the potential between 100 and 1200 mV and the pH between 4.0 and 7.6 (Figure 2). The optimum values of potential for each substance were: 1100 mV for 4-chlorophenyl urea, 900 mV for 4-chloroaniline and 700 mV for internal standard. A potential of 1100 mV was selected because lower potentials diminished the 4-chlorophenyl urea signal. On the other hand, an optimum value of 7.0 was selected for the analytical work.

The effect of the buffer molarity on the height of the peak was examined at pH=7.0 using phosphate/citrate buffer ranging from 0.005 to 0.05 mol l^{-1} in citric acid. It was observed that peak height increases when the ionic strength increases. Nevertheless, an increase of background was also observed. That is why an optimum value of the buffer molarity that guarantees a stable measure value, 0.005 mol l^{-1}, was selected.

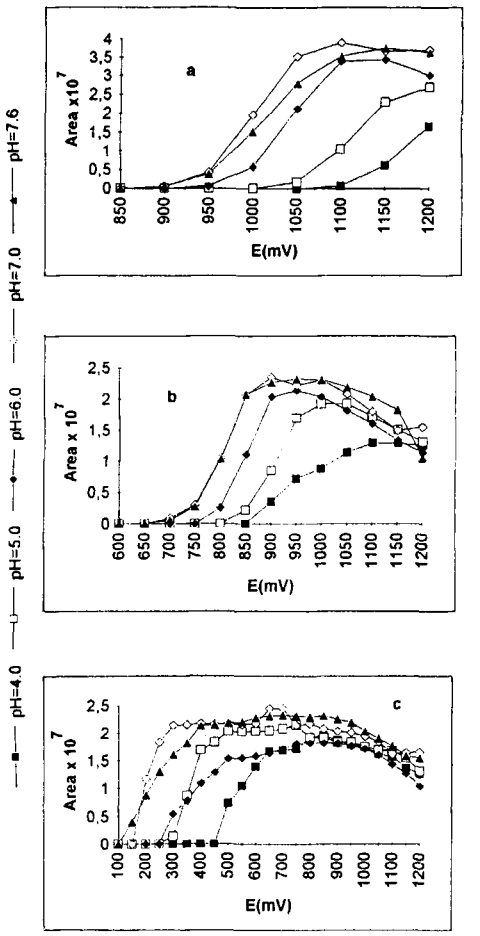

Figure 2. Hydrodynamic voltammograms. (a) 4-chlorophenyl urea 1.0 mg l^{-1}, (b) 4-chloroaniline 0.5 mg l^{-1}, (c) todralazine 1.0 mg l^{-1}. Injection volume: 20 µL. Flow rate: 1 mL min^{-1}.

Two organic modifiers, methanol and acetonitrile or, a mixture of them, were used to optimize the time of analysis, the peak width and the resolution. Table 1 shows the influence of the acetonitrile and methanol contents of the mobile phase in relation to the capacity factor K'.

Acetonitrile produced better defined chromatographic peaks than methanol, with less peak broadening. The best result for those three compounds was obtained when a mixture of acetonitrile-buffer (30:70) with a

Table 1

Influence of the Organic Modifiers Content of the Mobile Phase in Relation to K′

CH_3CN (%)	CH_3OH (%)	Citric Acid Phosphate Buffer (%)	K'_{tod}	$K_{chloroph}$	$K'_{chloraniline}$
20	0	80	4.32	6.16	12.70
20	10	70	3.14	4.70	8.53
25	0	75	1.98	2.93	6.89
25	5	70	2.46	3.50	6.88
25	10	65	1.87	2.71	5.15
28	0	72	1.95	2.90	6.82
30	0	70	1.44	2.43	5.80
30	5	65	1.41	2.21	4.57
32	0	68	1.32	2.19	5.27
35	0	65	0.42	1.74	4.54
0	40	60	3.80	5.77	8.94
0	50	50	1.90	2.84	5.47

Table 2

Mean Recoveries of 4-Chlorophenil Urea and 4-Chloroaniline in Different Solid Extraction Phases (n = 5)

Solid Phase	4-Chlorophenyl Urea	4-Chloroaniline
Aminopropyl	99.4 ± 1.9	95.5 ± 3.1
Cyanopropil	95.0 ± 2.1	90.8 ± 2.9
Florisil	95.3 ± 1.5	85.6 ± 1.1

flow rate of 1.0 mL min^{-1} was used. The retention times obtained for todralazine, 4-chlorophenyl urea and 4-chloroaniline were 2.11, 3.91 and 7.81 min, respectively (Figure 3). The detector used in this work is based on the principle of a thin-layer cell. Because the mass transport to the electrode is controlled by both diffusion and convection, the dependence of the detector response on flow rate must be studied. As was expected, the peak area decreased with an increase in flow rate, while the effect on the resolution was

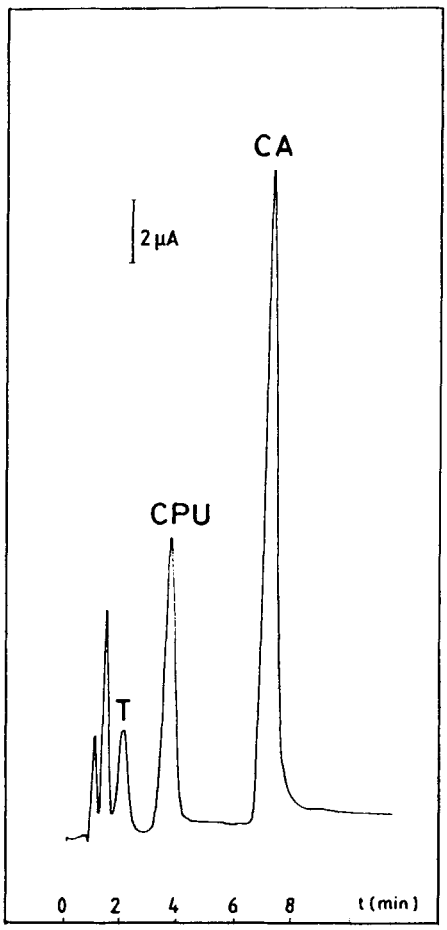

Figure 3. Chromatogram of 4-chlorophenyl urea (CPU) and 4-chloroaniline (CA) in optimum conditions: flow rate: 1.0 mL min-1, E=1100 mV, acetonitrile-citrate buffer (30:70), pH=7.0. Injection volume: 20 µL. Concentration: 200 µg l^{-1}. Internal standard: todralazine (T), 200 mg l^{-1}.

practically negiglible. A value of 1 mL min^{-1} was chosen as optimum. The determination of these compounds is based on the linear dependence of the relationship "$area_{compound}/area_{internal\ standard}$" on the concentration of the substance (in ng). A linear response was observed for concentrations ranging from 0.01 to 12.00 ng. The linear calibration plots correspond to the equations:

$A_{4\text{-chlorophen}} / A_{is} = 0.0785 + 0.0142$ [4-chlorophenyl urea],

$r = 0.9987$

$A_{4\text{-chloroanil}} / A_{is} = 0.0414 + 0.0325$ [4-chloroaniline],

$r = 0.9992$

The limits of detection based on 2N/m ratio (N is the noise and m is the slope of calibration graph) were found to be 9.2 pg for 4-chlorophenyl urea and 4.0 pg for 4-chloroaniline.

The relative standard deviations (n=10) at a concentration level of 0.16 ng (8 ppb) injected were 4.50 and 3.93%. These values decreased to 1.24 and 3.93% when 8.00 ng (400 ppb) of each substance were injected.

Analytical Applications

The validity of the proposed method was proven by spiking with 4-chlorophenyl urea and 4-chloroaniline in honey samples over the range of 10 to 60 mg Kg^{-1} and subjecting the samples to the described analytical procedure.

The prior extraction of the two substances from honey was established using different solid-liquid extraction polar phases: florisil, aminopropyl and cyanopropyl. The recovery data are reported in Table 2. The best resolution from extracts was obtained using aminopropyl cartridge in the clean-up stage. No significative differences in the extraction efficiency were observed when the concentration levels varied in the studied range.

Figure 4 illustrates the chromatograms for a blank sample honey and a spiked sample honey containing 25 $\mu g\ Kg^{-1}$ of 4-chlorophenyl urea and 4-chloroaniline. The high mean recovery obtained permitted the establishment of low detection limits.

The method can be applied to the determination of 4-chlorophenyl urea and 4-chloroaniline in honey at levels of 0.23 and 0.10 $\mu g\ Kg^{-1}$ respectively.

In conclusion, the HPLC-ED method developed has been used for the sensitive and reproducible determination of the major diflubenzuron metabolites. 4-chlorophenyl urea and 4-chloroaniline. The low detection limit achieved makes the monitoring of both compounds in honey samples possible.

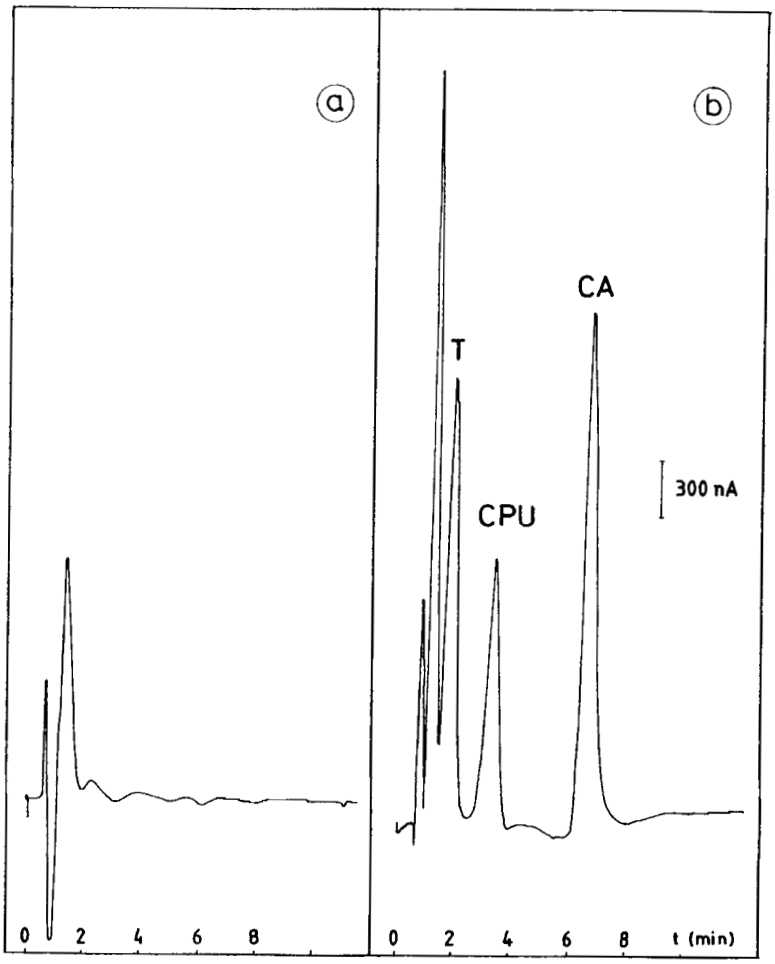

Figure 4. (a) Blank honey, (b) Chromatogram of fortified honey sample extract with the usual doses: 25 mg kg^{-1} of 4-chlorophenyl urea and 4-chloroaniline, internal standard injected: 200 μg l^{-1}. Other conditions in Figure 3.

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REFERENCES

1. A. Retnakaran, J.E. Wright, "Control of Insect Pest with Benzoylphenyl Ureas" in **Chitin and Benzoylphenyl Ureas**, J. E. Wright, A. Retnakaran, eds., Dr.W.Junk Publishers, Dordrecht., The Netherlands, 1987, pp. 205-282.

2. A. Retnakaran, A. G. Raske, R. J. West, K. P. Lim, A. Sundaram, J. Econ. Entomol., **81**, 1698-1705 (1988).

3. S. A. Fischer, L. W. Hall, Crit. Rev. Toxicol., **15**, 1426-1435 (1992).

4. L. S. Mian, M. S. Mulla, Residue Rev., **84**, 27-112 (1982).

5. K. A. Smith, A. A. Girgarick, J. H. Lynch, J. Econ. Entomol., **78**, 185-189 (1985).

6. G. W. Ivie, D. L. Bull, J. A. Veech, J. Agric. Food. Chem., **28**, 330-337 (1980).

7. C. H. Schaefer, E. F. Dupras, J. Agric. Food. Chem., **24**, 733-739 (1976).

8. A. De Kok, M. Van Opstal, T. De Yong, B. Hoogcarspel, R. B. Geerdink, R. W. Frei, U. A. Brinkman, Intern. J. Environ. Anal. Chem., **18**, 101-106 (1984).

9. U. A. Brinkman, A. De Kok, R. B.Geerdink, J. Chromatogr., **283**, 113-116 (1984).

10. H. B. Lee, J. Chromatogr., **457**, 267-270 (1988).

11. M. Hoble, C. Stern, C. Asovata, K. Williams, J. Chromatogr. Sci., **29**, 131-135 (1991).

12. R. C. C. Wegman, G. A. L. De Korte, Intern. J. Environ. Anal. Chem., **9**, 1-6 (1981).

13. A. De Kok, Y. J. Vos, C. Van Garderer, T. De Jong, M. Van Opstal, R. W. Frei, R. B. Geerdink, U. A. T. Brinkman, J. Chromatogr., **288**, 71-90 (1984).

14. G. Boër, C. Schlett, H. P. Thier, Z. Abwasser, Forsch., **23**, 220-223 (1990).

15. E. M. Lores, D. W. Bristol, R. F. Moseman, J. Chromatogr. Sci., **16**, 358-362 (1978).

16. M. S. Varney, M. R. Preston, J. Chromatogr., **348**, 265-272 (1980).

17. E. M. Lores, F. C. Meekins, R. F. Moseman, J. Chromatogr.,**188**, 412-416 (1980).

18. A. Di Corcia, R. Samperi, Anal. Chem., **62**, 1490-1494 (1990).

19. S. Hatrik, J. Lehotay, J. Tekel, J. High. Resol. Chromatogr., **17**, 756-758 (1994).

20. P. Cabezuelo, J. Fernandez, J. Romero, F. Prats, Vida Apícola, **77**, 45-48 (1996)

21. M. A. Fernandez, M. J. Riol, C. Herrero, M. I. García, Vet. Hum. Tox., **36**, 429-432 (1994)

22. W. van Rillaer, H. Beernaert, Z. Lebensm. Unters. Forsch, **188**, 135-137 (1989)

23. P. Cabras, M. Melis, L. Spanneda, J. AOAC. Int., **76**, 92-94 (1993).

24. W. Maae, R. Vanhes, A. C. Grosscurt, D. H. Deul, **Benzoylphenylurea Insecticides in Chemie**, Wegher R., ed., Springer-Verlag, 1980.

25. P. R. Gupta, R. S. Chandel, Apidologie, **26**, 3-10 (1995).

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