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Capillary gas chromatography with mass spectrometric and atomic emission detection for characterization and monitoring chlordimeform degradation in honey☆

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Abstract

Capillary gas chromatography–mass spectrometry and capillary gas chromatography–atomic emission detection have been successfully used to identify and monitor the main degradation products of chlordimeform when this compound is initially present in honey. The analysis of laboratory-spiked honey samples over 28 weeks revealed the occurrence of two degradation products: 4-chloro-*o*-toluidine (**I**) and *N*-formyl-4-chloro-*o*-toluidine (**III**). During this period the concentration of chlordimeform decreased to 7.5% of its initial value; the concentration of compound **I** increased gradually whereas compound **III** was present in a larger proportion and reached a maximum around the 14th week.

Keywords: Chlordimeform; Chlorotoluidines; Toluidines; Pesticides

1. Introduction

Chlordimeform, *N*-(4-chloro-*o*-tolyl)-*N*,*N*-dimethylformamidine (**II**), is an acaricide which is used in some countries [1] against the mite *Varroa jacobsoni* Oud, which seriously affects honey beehives. In some cases, probably due to poor application of the compound, it is possible that residues can appear in honey and, that during storage, degradation products can develop, so methods are necessary for the detection and quantitation of all compounds involved. Chlordimeform analysis in honey samples, either alone or in combination with other acaricides, has been reported in several papers. In most cases, gas chromatography is used for its determination in extracts [2–6]. This technique has also been proposed for determining three degradation products of chlordimeform: 4-chloro-*o*-toluidine, *N*-formyl-4-chloro-*o*-toluidine and 2,2′-dimethyl-4,4′-dichlorobenzene, in other matrixes, such as rice cargo and husk [7].

The degradation of chlordimeform, and the identification of related transformation compounds in water samples kept under different conditions in the laboratory, has also been studied by GC and HPLC

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[8]. Various degradation products have been reported in vegetables, insects and mice, and some degradation pathways have been proposed [9]. As far as we known, there is no information about the degradation and persistence of chlordimeform and possible degradation products in apiarian products, particularly honey.

In this work, degradation products of chlordimeform are detected by gas chromatography, and identified after conventional solvent extraction of spiked honey samples. The characterization and identification are based on mass spectrometry and atomic emission detection coupled on-line with capillary gas chromatography. It is well known that the complementary use of both techniques can be helpful to solve many problems in the identification of microcontaminants and their degradation products in several matrices [10–16]. For this reason, we thought that they could also be used to investigate the behavior of chlordimeform, in case it was present in a honey sample.

2. Experimental

2.1. Reagents

Residue analysis grade hexane and acetone were supplied by Scharlau (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q plus apparatus (Millipore, Milford, MA, USA). Chlordimeform and chlorpyrifos certified standards were purchased from Promochem (Wesel, Germany). Sodium hydroxide was obtained from Panreac (Barcelona, Spain).

2.2. Preparation of spiked samples

A multifloral honey sample (150 g) was heated in a water bath at 35 °C for 15 min, and allowed to stand for 5 min. Then 2 ml of a suspension of chlordimeform in water were added to the honey. The mixture was mechanically stirred with a blender in order to ensure homogenization and then transferred to a glass container. The container was closed and kept in a refrigerator. To identify the degradation products, a spiking level of 100 mg/kg was used, and to monitor the evolution of chlordimeform and its degradation products, the spiking level was 2 mg/kg.

2.3. Extraction

The extraction procedure used to isolate the target compounds was a simplification of a method previously published [5], and devoted to the analysis of acaricides in honey samples. In this work, the clean-up step has been omitted. An amount of honey (1 g) was mixed with 1 ml of water at pH 9, and the mixture was extracted twice with 25 ml of an *n*-hexane–acetone (8:2, v/v) mixture, under mechanical stirring for 30 min. The extract was separated by centrifugation at 2500 *g* for 10 min. The liquid phases were combined and evaporated to dryness, at room temperature, under a gentle nitrogen stream. Finally, the extract was dissolved in 1 ml of *n*-hexane and passed through a PTFE filter of 0.50-μm pore size, prior to chromatographic analysis.

2.4. Chromatographic analysis

A Hewlett-Packard 6890 gas chromatograph (Little Falls Site, Wilmington, DE, USA), was directly coupled to a Hewlett-Packard 5973 mass spectrometer. The chromatograph was fitted with a 30 m×0.25 mm, 0.25 μm DB-17 column from J&W Scientific (Folsom, CA, USA). The oven temperature was kept at 50 °C for 1 min and then programmed at 5 °C/min to 275 °C, then held there for 8 min. The carrier gas (helium) pressure program was as follows: initial pressure 20.7 MPa, a ramp at 682.6 MPa/min to 275.8 MPa (0.2 min), a ramp at 682.6 MPa/min to 34.5 MPa, and then a ramp at 2.3 MPa/min to 137.9 MPa (5 min). Splitless injection (5 μl) was performed with an HP 7673A automatic sampler at an injection port temperature of 200 °C; the purge valve was on at 1 min; the transfer line temperature was 280 °C. The MS temperatures were as follows: ion source 200 °C, quadrupole 100 °C. Positive (PCI) and negative (NCI) chemical ionization was performed with methane as a reagent gas. Electron multiplier voltage was maintained 200 V above autotune. The scan range was 50–450 u.

The other system consisted of a Hewlett-Packard 5980 Series II chromatograph, an HP 7673A automatic sampler, and an HP 5921A atomic emission

detection (AED) system. GC–AED was performed on a 30 m×0.25 mm, 0.25 μm DB-17 column (J&W Scientific). The oven temperature was kept at 50 °C for 1 min and then programmed at 5 °C/min to 275 °C and held there for 8 min. The carrier gas (helium) pressure was as follows: initial pressure 48.2 MPa, a ramp at 413.7 MPa/min to 275.8 MPa (0.2 min), a ramp at 682.6 MPa/min to 48.2 MPa, and a ramp at 2.6 MPa/min to 172.4 MPa (3 min). Splitless injection (3–5 μl) was performed at 200 °C, and the purge valve was on at 1 min. The transfer line and cavity temperatures were 280 °C; the make-up flow (He) was 100 ml/min. The elements analyzed and their emission lines, in nanometers, were: carbon 193, hydrogen 486, nitrogen 174, oxygen 777 and chlorine 479.

3. Results and discussion

3.1. Identification of transformation products by GC–AED and GC–MS

Honey samples spiked with high concentrations of acaricide, 100 mg/kg, and kept at room temperature for a month, were used in order to identify the degradation products, using a procedure similar to the one used in previous studies [10,11].

The extracts obtained from these samples were injected in the GC–AED system. Their analysis revealed the occurrence of three nitrogen and chlorine-containing compounds (Fig. 1). The symbols, **I, II** and **II,** have been assigned according to their retention times. One of the compounds was chlordimeform (**II**). The presence of chlorine in the compounds was corroborated by observation of its characteristic emission wavelengths: 479, 481 and 482 nm, whose intensity decreased in the same order. The molar elemental ratio of the detected compounds was calculated by using chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$) as a calibration compound (Table 1); this compound was added to the injected extracts to reduce error. The retention time of chlorpyrifos in the GC–AED system was 36.82 min.

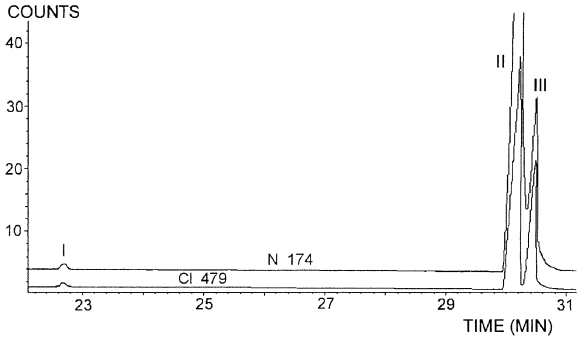

Fig. 1. Chromatogram of a honey extract obtained by nitrogen and chlorine emission lines from the atomic emission detector. Chlordimeform fortification level: 100 mg/kg.

The sensitivity achieved using the atomic emission lines was also studied. Data were obtained by the injection of chlordimeform standards using a signal-to-noise ratio of 3; the detection limits were observed for 3 non-consecutive days. The nitrogen (174 nm) and chlorine (479 nm) lines were less sensitive, with detection limits of 50 and 350 μg/l, respectively, than the carbon (193 nm) line, whose detection limit was 1 μg/l. For that reason, samples spiked with high acaricide levels are recommended for the search of chlordimeform related compounds. By mass spectrometry the detection limits in electron impact were 1000 μg/l in scan mode and 20 μg/l in the selected ion monitoring (SIM) mode (target ion 196, qualifier ions 117 and 181). In NCI (target ion 195, qualifier ion 197) the detection limit was ~1 μg/l.

Injection of the extracts in GC–MS in the electron impact ionization (EI) mode allowed identification of compound **I** (Fig. 2) with the help of spectrum libraries. Compound **III,** which eluted very close to chlordimeform, was readily identified by detecting a mono-chlorinated ion fragment at *m*/*z* 169. This fragment was the molecular ion, as confirmed by PCI-MS. Moreover, a loss of 29 u (assigned to a formyl group, –CHO) from the ion *m*/*z* 169 was

Table 1
Elemental molar ratio and estimated empirical formulae for chlordimeform and its two transformation products (average of five determinations)

Peak	Elemental ratio	Estimated formula	Real formula
I	$C_1H_{1.302}N_{0.109}Cl_{0.114}$	$C_7\ H_{9.1}\ N_{0.8}Cl_{0.8}$	$C_7\ H_8\ NCl$
II	$C_1H_{1.370}N_{0.227}Cl_{0.122}$	$C_{10}H_{13.7}N_{2.3}Cl_{1.2}$	$C_{10}H_{13}N_2Cl$
III[a]	$C_1H_{1.150}N_{0.099}Cl_{0.096}$	$C_8\ H_{9.2}\ N_{0.8}Cl_{0.8}$	$C_8\ H_8\ NClO$

[a] Oxygen not detected by GC–AED.

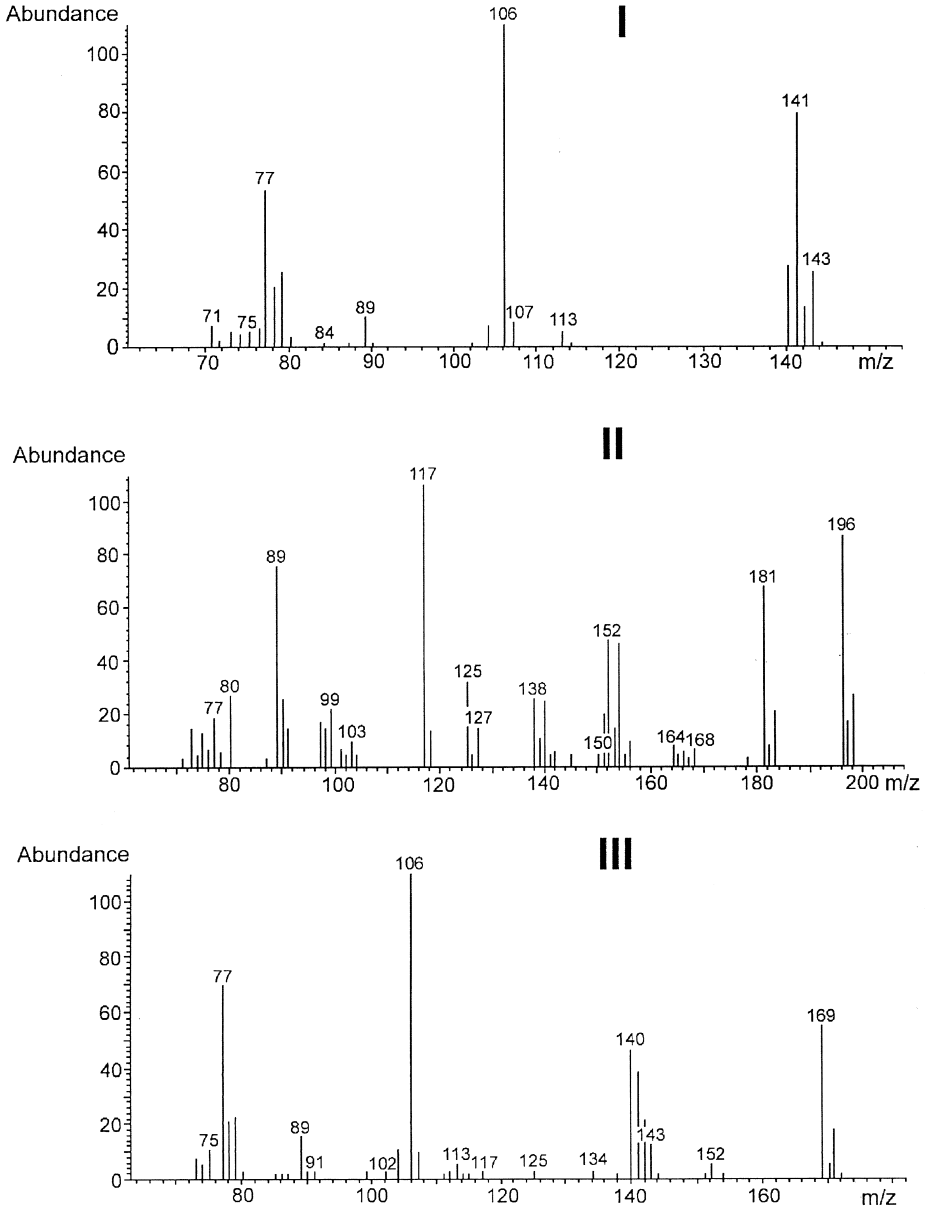

Fig. 2. Electron impact spectra of the compounds.

observed. These data are indicative of a well-known degradation product of chlordimeform: *N*-formyl-4-chloro-*o*-toluidine.

The structures of the compounds were confirmed by GC–MS in the PCI mode and with the data provided by GC–AED. In Fig. 3, the ions $[M+1]^+$ and $[M+29]^+$ for the three related compounds can be seen. The empirical formulae estimated from the elemental molar ratio and the molecular masses obtained by MS (PCI) are shown in Table 1; they were, in general terms, consistent with the real formulae of the compounds. The highest relative errors for the N/C, Cl/C and H/C ratios were 23, 22 and 15%, respectively. It is interesting to note that

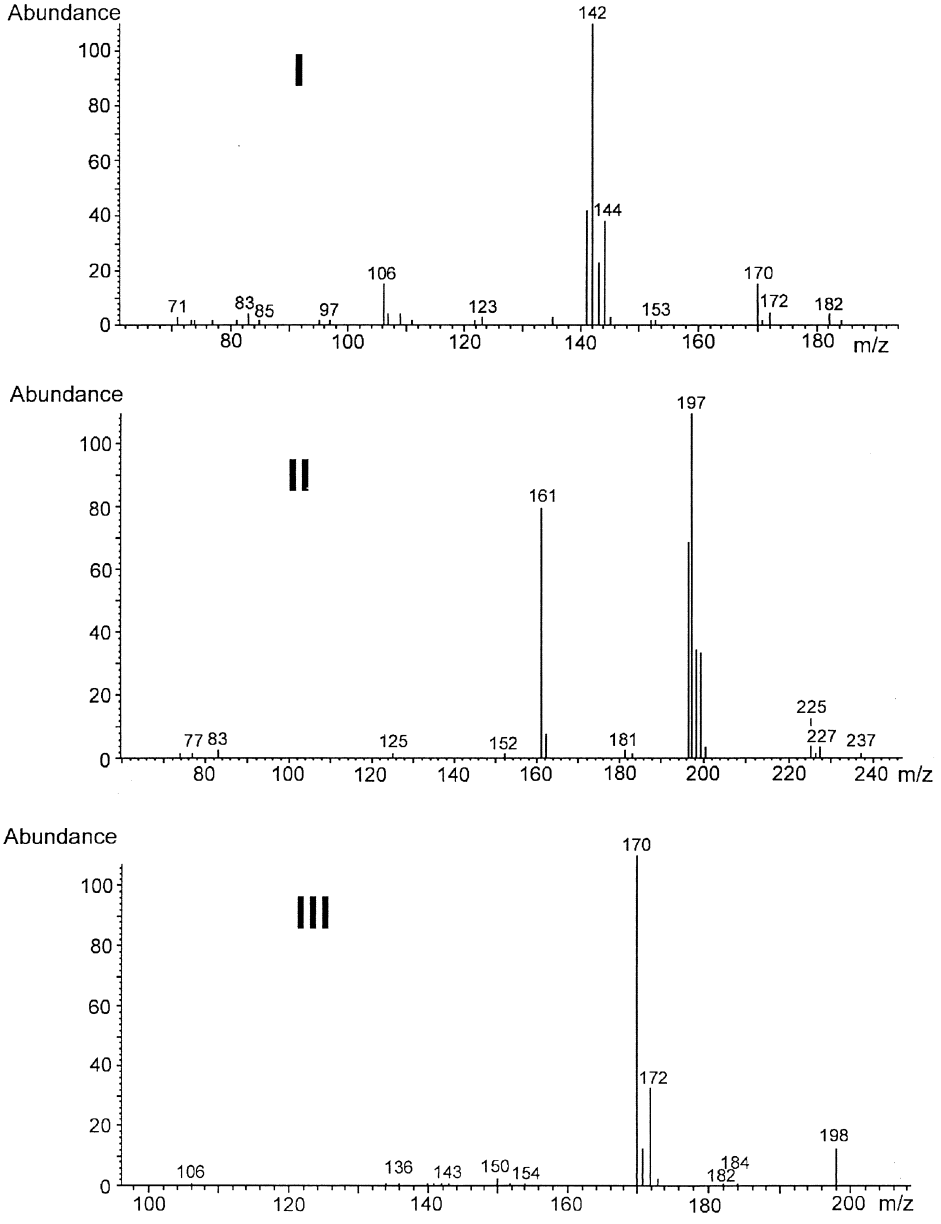

Fig. 3. Positive chemical ionization spectra of the compounds.

oxygen has not been detected, owing to its high detection limit. The NCI-MS spectra were also recorded, and, as expected, an increase in the sensitivity was observed, except for compound **I**.

Fig. 4 shows the structures elucidated for the degradation products. Table 2 lists their names, molecular ions and retention times in the two GC systems. The retention times obtained for the same compound in both GC systems were linearly correlated by the equation $t_{AED} = (0.92\ \ t_{MS}) + 3.47$, where t is the retention time. This equation makes their location in the chromatograms easier.

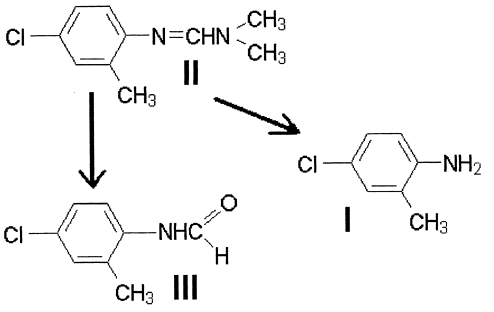

Fig. 4. Structure of chlordimeform and its two metabolites found in spiked honey.

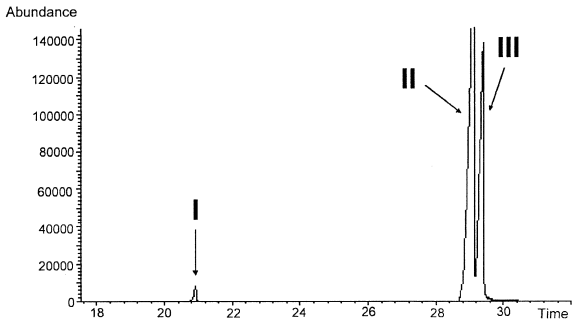

Fig. 5. Chromatogram of an extract of spiked honey, obtained by EI-MS in the SIM mode. Fragment-ions monitored: compound **I**: 106+141+143; compound **II**: 117+181+196; compound **III**: 106+140+169. Time scale in minutes.

3.2. Degradation of chlordimeform in honey

The degradation study was carried out on a multifloral honey sample spiked with chlordimeform at a concentration of 2 mg/kg. The sample aliquots were placed in a glass container and kept at room temperature (18–22 °C) avoiding direct exposure to sunlight.

Samples were taken weekly, taking three aliquots of 1 g, and were submitted to the proposed procedure. Chlorpyrifos was added to the extract, at a concentration level of 1 mg/l, to correct for instrumental variations. Degradation was mainly monitored through the GC–AED system at a wavelength of 193 nm, which corresponds to an emission line from the carbon atom. This emission line was preferred to the nitrogen and chlorine lines because of its higher sensitivity. The presence of compounds **I**, **II** and **III** was also confirmed by EI-MS in the SIM mode (Fig. 5).

The evolution of chlordimeform and its two degradation products, expressed in terms of peak area, is shown in Fig. 6. As can be seen, the concentration of chlordimeform decreases gradually: after 28 weeks it was 0.15 mg/kg (RSD 5.3%, $n=3$), 7.5% of its initial concentration. The amount of compound **I** increases during the first 12 weeks, until it reaches a value which remains virtually constant for the rest of the experiment. The concentration of compound **III** increases faster, reaching a maximum in the 14th week, diminishing slowly afterwards. It is interesting to note that these degra-

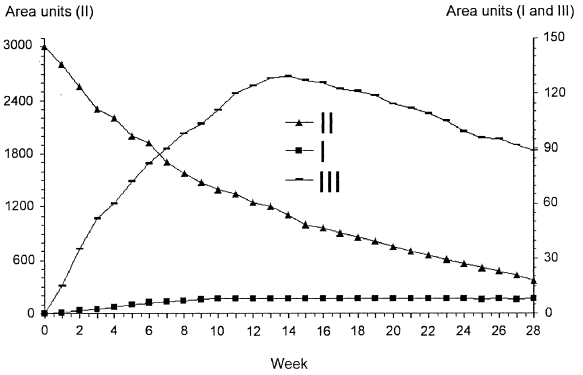

Fig. 6. Degradation of chlordimeform in honey and evolution of its metabolites with storage time.

Table 2
Retention times, molecular ions and names of the compounds

Peak	Retention time (min)		Molecular ion	Compound
	AED	MS		
I	22.69	20.90	141	4-Chloro-*o*-toluidine
II	30.22	29.06	196	*N′*-(4-Chloro-2-methylphenyl)-*N*,*N*-dimethylmethanimidamide
III	30.47	29.39	169	*N*-Formyl-4-chloro-*o*-toluidine

dation products are analogous to those found in the study of amitraz degradation in honey [11]. This is probably due to the similarity of the structures of both pesticides (formamidines). On the other hand, it is noticeable that the concentration of compound **I** was lower than we had expected because it is the most abundant degradation product of chlordimeform in animals, vegetal and environmental samples [17].

An estimation of the final concentrations (after 28 weeks from spiking) of the compounds **I** and **III** has been carried out using the carbon line of the AED system. For this purpose, a calibration graph has been made with the peak heights obtained for chlordimeform concentrations of 0.01, 0.05, 0.10, 0.25, 0.50, 0.75 and 1 mg/l, equivalent to carbon concentrations of 0.006–0.6 mg/l. The correlation coefficient (r^2) of the linear fitting was 0.990. Based on this, the concentrations of compounds **I** and **III** were 0.009 and 0.06 mg/kg, respectively. In this estimation, it is assumed that emission intensity was independent of the structure of the compounds. The RSDs of the estimations were 17.9% for compound **I** and 5.0% for compound **III** ($n=3$).

4. Conclusions

The combination of GC–MS and GC–AED facilitates the search and identification of transformation products of chlordimeform in honey. The selectivity of nitrogen and chlorine emission lines of the AED system simplifies the chromatograms of the honey extracts and allows a rapid screening of the structurally related compounds. Moreover, this detector corroborates the identity of the compounds, established by the MS spectra, through the estimation of their empirical formulae.

Chlordimeform degrades in honey. The progressive formation of two transformation products, whose kinetics of formation are very different, has also been observed. For an initial concentration of 2 mg/kg, the content of chlordimeform in the honey after 28 weeks is 0.15 mg/kg, whereas the concentration of 4-chloro-*o*-toluidine is ~0.009 mg/kg and that of *N*-formyl-4-chloro-*o*-toluidine is ~0.06 mg/kg, as deduced from a calibration based on the carbon emission line.

Acknowledgements

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