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Identification of diflubenzuron by packed-capillary supercritical fluid chromatography–mass spectrometry with electron-capture negative ionization

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Abstract

The thermolabile insecticide diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] was successfully chromatographed by supercritical fluid chromatography on a 320 μm I.D. Inertsil C_8 column using neat carbon dioxide as mobile phase.

Detection and electron-capture negative ionization (ECNI) mass spectrometric characterization were accomplished using a double focusing mass spectrometer with a modified ion source and coaxial direct fluid introduction. Detection limits of 1.0 μg/ml and 0.03 μg/ml were obtained in full-scan and selected-ion monitoring (SIM) mode, respectively. The restrictor heater temperature was found to have a major influence on the signal intensity and fragmentation pattern, as thermal degradation was believed to take place or be initiated on the hot restrictor wall. The relative abundance of the molecular ion amounted to 100% using restrictor temperatures of 160°C and 170°C, while it was completely absent at 190°C. At the lower temperatures, $[M - HF]^{-\bullet}$ was the most significant fragment ion.

1. Introduction

Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea] is a thermolabile insect growth regulator [1] and direct determination by gas chromatography is impossible due to thermal degradation [2]. The methods available for direct quantitation are based upon high-performance liquid chromatography (HPLC) with UV detection [3]. However, due to the non-specific detection, long clean-up procedures are often required. Gas chromatographic methods including derivatization and subsequent electron capture, as well as nitrogen–phosphorus or mass spectrometric detection have been used for quantification [4–6]. Diflubenzuron has also been quantified by a GC–MS method using deuterated diflubenzuron as internal standard, by quantitation of the heat decomposition products [2].

However, methods including direct compound identification as well as quantitation are preferable. Supercritical fluid chromatography (SFC) has been recognized as a potential technique for the determination of thermolabile pesticides [7–9]. The combination SFC–MS has been shown to be applicable for the identification of different pesticides [10–13]. This work focuses primarily upon the possibility of using SFC–MS for the determination of diflubenzuron. Due to the limitations of the MS, relatively small volumetric flow-rates are allowed in the SFC system. Thus, packed capillary columns appear to be the col-

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umn of choice because of their higher sample loadability as compared to open capillary columns. High sample loadability is important since low concentrations are expected to be present in real samples. Determination of diflubenzuron in real samples usually requires a sample preparation step including preconcentration and purification; however, this was not the subject of our work.

The halogen-containing diflubenzuron should be applicable to negative-ion detection in the MS. The rate constant for ion formation in EC depends strongly on the collision cross-section and can exceed 10^{-7} $cm^3 s^{-1}$ for polyhalogenated compounds [14]. In such cases, sensitivity might be 100 times better as compared to positive chemical ionization (PCI). ECNI was therefore our choice for detection.

Since the standard ion source for electron ionization (EI) and chemical ionization (CI) was found to be unsatisfactory for negative-ion detection [13], the second aim of this study was to design a better interface–ion source system for negative-ion detection in SFC–MS

2. Experimental

2.1. Chemicals

Both packing and chromatography were performed using neat CO_2 (99.998%) supplied by Aga Norgas (Oslo, Norway). The solvents used were CS_2 (>99.0%) from J.T. Baker Chemicals (Deventer, Netherlands), HPLC grade tetrahydrofuran (THF) (Rathburn, Walkerburn, UK) and pro-analysis grade formamide (Merck, Darmstadt, Germany). The THF was filtered through aluminium oxide prior to use. Potassium silicate solution was purchased from Kebo Lab. (Oslo, Norway). Analytical grade diflubenzuron was obtained from the Agricultural University of Norway (Aas, Norway) and dissolved in THF or CS_2. MS-calibration was carried out using low boiling perfluorokerosene (PFK) (Tokyo Kasei, Japan).

2.2. Columns and restrictors

Fused-silica 320 μm I.D. and 450 μm O.D. from Polymicro Tech. (Composite Metal Services, UK) was used for the columns. The packing material was 5 μm silica-based Inertsil C_8 (Keystone, USA) or 4 μm Nova-Pak C_{18} (Waters, USA). Both columns were 60 cm long and prepared in the laboratory, according to the method described by Malik et al. [15]. A 2-mm ceramic frit was used at the outlet of the column. Valco zero dead volume unions (1/16 in. to 1/16 in.) and one piece fused-silica adapter ferrules were utilized for column fitting.

The integral restrictor [16] was made from fused-silica capillary (I.D. 50 μm, O.D. 365 μm). For the purpose of fast analyses, a flow was chosen that gave a t_0 value of ca. 3–4 min at 100 bar of pump pressure.

2.3. Instrumentation

Supercritical fluid chromatography was performed with a Model 602 system from Lee Scientific (Salt Lake City, UT, USA). A CI4W injector from Valco Instruments (Houston, TX, USA), equipped with a 0.2-μl loop, was used with timed split injection at room temperature. A small piece of fused-silica capillary (I.D. 50 μm) was used to connect the column to the injector. In order to prevent backflushing of packing material, a small stainless-steel frit (No. 24000, Keystone Scientific) was placed in the union connected to the column entrance. The whole column was kept in the oven at a constant temperature of 60°C. The restrictor was attached directly to the column end union.

The mass spectrometer used for detection was a JMS-DX303 from JEOL (Tokyo, Japan). It is a double focusing instrument with EB-geometry. Ion detection was done by the ion multiplier equipped with a 10-kV post-acceleration conversion dynode unit. Detector sensitivity adjustments were kept constant while performing these experiments, making it possible to compare results. The ion source pressure, as referred to in this work, was actually the pressure measured by

a gauge placed some distance from the ion source. The pressure inside the ion source was considerably higher, since the ion source was made as small and sealed as possible. Cryopumping was used in all experiments. The modified CI ion source, made at the institute, had a channel drilled through its metal block. To achieve cooling, nitrogen gas, at room temperature and slightly above ambient pressure, was led through this channel. An even lower ion source temperature was reached by precooling the nitrogen in a copper tube placed in a mixture of methanol and dry ice at −77°C.

Direct fluid introduction (DFI) was made possible by designing a simple restrictor heating interface (Fig. 1), consisting of a 4-cm steel tubing (I.D. 1.0 mm) containing both the restrictor and an insulated heater. Two pieces of fused-silica capillary were used to insulate the thermowire applied as heater. The fluid was allowed to enter the ion source coaxially, toward the analyzer, by making the DFI-interface a part of the repeller (Fig. 2). The ion source side entrance was plugged with a Teflon-insulated thermocouple, making accurate ion source temperature measurements possible. The thermocouple tip was placed less than 1 mm inside the ion source. The LC controller of the MS supplied the electric current for the heater and restrictor temperature monitoring (at the outside of the stainless-steel tubing). The heat from the filament generally kept a stable ion source temperature of 150°C, when no cooling gas was added.

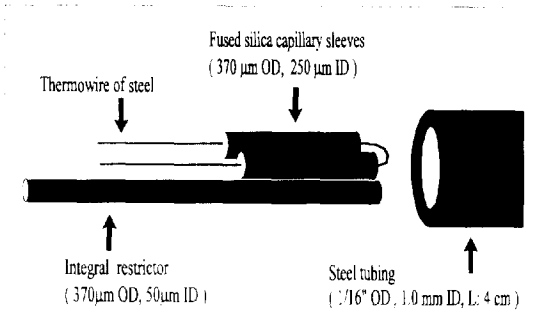


Fig. 1. Restrictor heater.

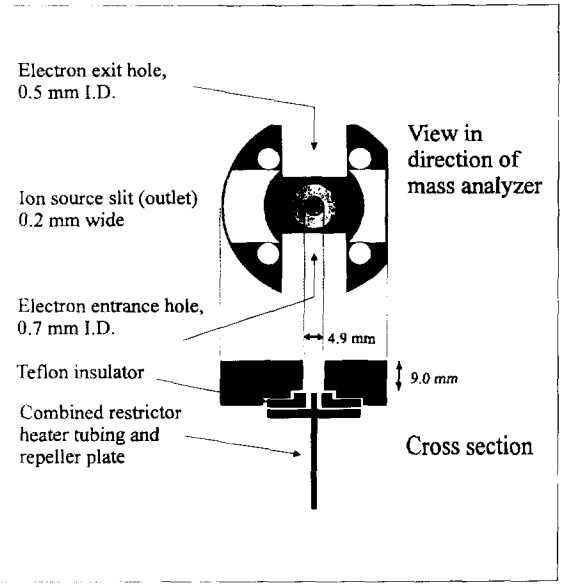


Fig. 2. Modified ion source (not to scale).

A rhenium filament was used, despite the background ReO_3^- and ReO_4^- ions associated with it. Rhenium was preferred instead of thoriated irridium [13], because of its relative low cost and ease of replacement. The internal diameter of the electron entrance hole was increased from 0.5 to 0.7 mm in order to make filament positioning less critical. The effect of this modification on detector sensitivity was not examined. A 100-μA ionization current was set, and electrons accelerated through 230 V. The repeller voltage was kept at 0 V.

The resolution, at 25% valley, was kept at 500 throughout. The cycle and scan times were set to 2.3 and 2.0 s, respectively, with a m/z range of 10 to 500 in full-scan mode.

3. Results and discussion

3.1. Chromatography.

Since diflubenzuron is capable of forming hydrogen bonds with free silanol groups, an inert packing material was sought. An inert packing material is also of importance when analyzing

real samples containing other components which can adsorb irreversibly to silanol groups and change the stationary phase characteristics. The highly endcapped C_8 Inertsil and Nova-Pak C_{18} materials were examined for the determination of diflubenzuron. As shown in Fig. 3, the chromatography of diflubenzuron on the C_8 Inertsil material is acceptable, avoiding the need for a modified CO_2 mobile phase. In fact, the addition of 5% (w/w) methanol to the CO_2, resulted in up to 100-fold reduction of the MS detection sensitivity for some halogenated compounds. A reduction was also observed for diflubenzuron. The Nova-Pak C_{18} material also seemed to be sufficiently deactivated for chromatography of diflubenzuron with neat CO_2. The C_8 group on Inertsil provided a decrease in retention compared to the Nova-Pak C_{18} material (data not shown), leading to shorter analysis time and elution at a lower pressure and was therefore preferred.

Using SFC with flame ionization detection an acceptable reduced plate height of about 7 was obtained for anthracene at k values of 4–5 on these columns. Evaluation of columns prepared by this method has been done recently by Tong et al. [17]. They have reported a reduced plate height as low as 2.33. This indicates the potential of such columns for separation of complex mixtures. As we did, they also found the columns to be very stable. No void volume was observed at the column entrance, even after months of use.

With the high flow-rate required to obtain fast elution on these columns, the ion source pressure was higher than the optimum value, which was about $5 \cdot 10^{-6}$ Torr at the gauge.

3.2. Detection

Preliminary experiments included cooling of the modified ion source to study the effect of temperature upon signal response since lower temperatures were previously shown to be beneficial for the detection of some chlorinated pesticides [13]. However with 1-chloroanthracene, 9,10-dichloroanthracene, hexachlorobenzene and 2,3-dichloronaphtoquinone a small decrease in peak heights measured on the molecular ion RIC (reconstructed ion current) chromatogram was observed, when reducing the ion source temperature from 150° to 100°C (data not shown). The detection of diflubenzuron was therefore performed at 150°C, since lowering the ion source temperature was not believed to seriously affect sensitivity and since cooling was avoided.

The modified ion source also provided the possibility of comparing the effect on signal response of two different introduction angles, either from the left side or coaxially. When introducing the fluid coaxially, toward the ana-

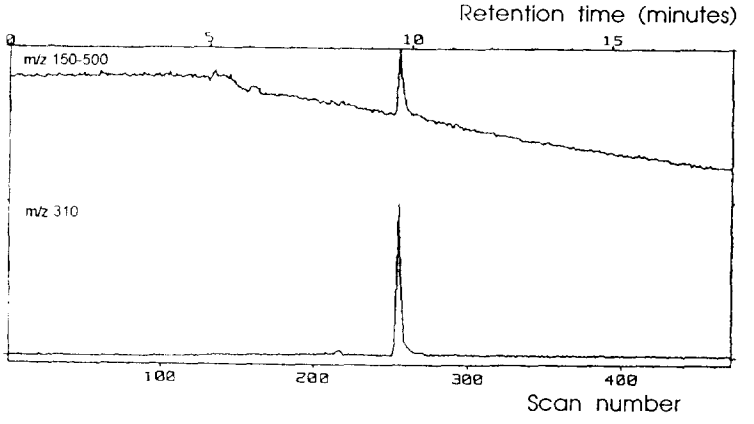


Fig. 3. RIC chromatograms of diflubenzuron on 5 μm Inertsil C_8 with EC detection. About 50 nl of a 0.05 mg/ml solution was injected. SFC conditions: column temperature 60°C and pressure programming 100 bar (3 min), then increased from 100 bar to 250 bar at 10 bar/min (linear gradient). MS conditions, see text. Restrictor temperature 160°C.

lyzer, increased molecular ion signals were generally found for the halogenated compounds investigated. This was therefore the preferred method.

A sufficient heating of the restrictor is vital for the success of any SFC system, since it prevents restrictor plugging and gives a stable flow. The mass spectrum of diflubenzuron in ECNI–MS turned out to be quite dependent on the restrictor temperature. The mass spectra at restrictor temperatures of 190°C (Fig. 4a) and 170°C (Fig. 4b) revealed a large degree of fragmentation in the former case and the molecular ion (m/z 310) was completely absent at 190°C. However, some of these fragment ions were suspected to be due to thermal degradation products.

Pathways for the thermal degradation of diflubenzuron have been reported in the literature [2]. Some of these thermal degradation products were detected by EC using a restrictor temperature of 190°C (Fig. 4a); the m/z 156 and 157 ions can be assigned to difluorobenzamide. The m/z 156 was the most abundant of the two and would likely be due to detachment of a hydrogen radical. The ion m/z 183, present at a lower abundance, probably corresponds to 2,6-difluorobenzoyl-isocyanate. Ions corresponding to the thermal degradation products 4-chloroaniline and 4-chlorophenylisocyanate were not observed, perhaps as a result of chloride anion detachment, as indicated by m/z 35/37.

The fragment ion $[M-HF]^{-\bullet}$ at m/z 290

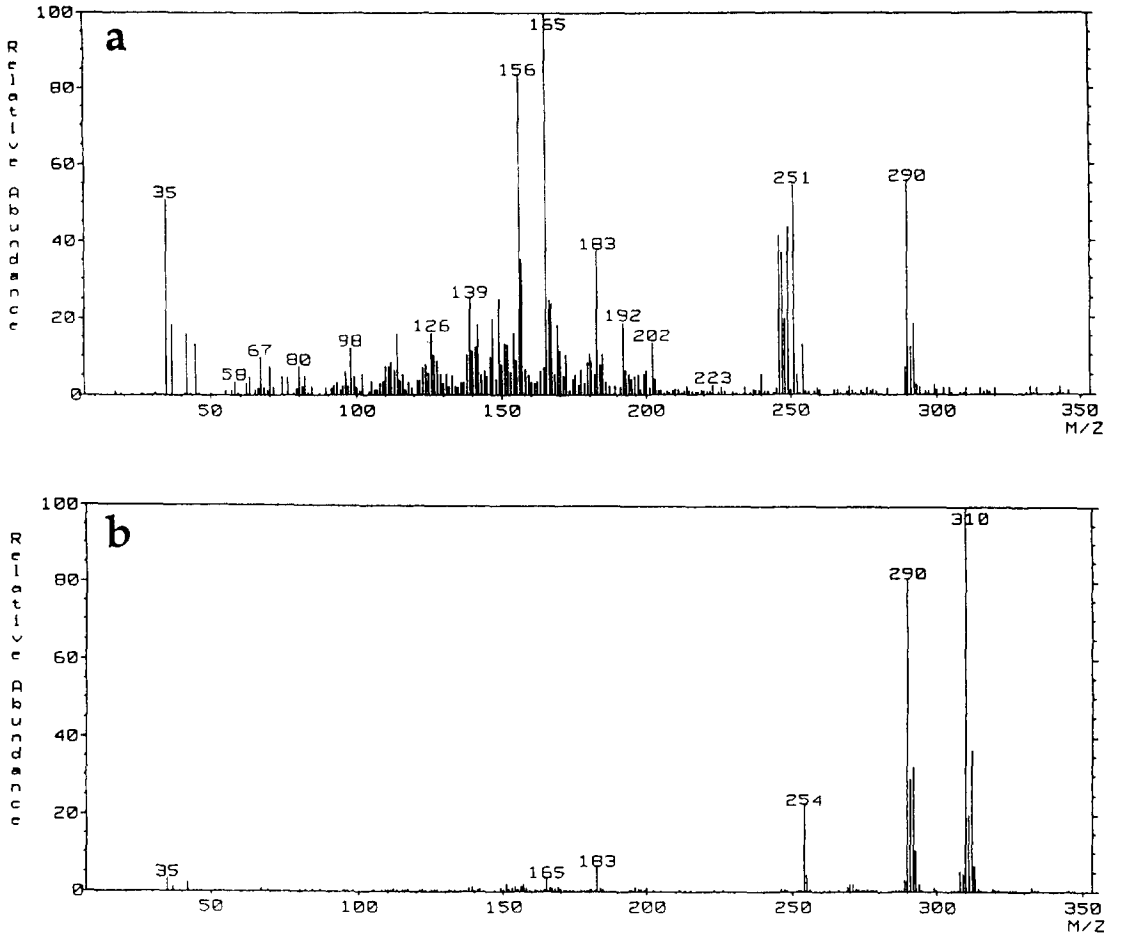


Fig. 4. The mass spectra of diflubenzuron at restrictor temperature of (a) 190°C, and (b) 170°C.

results from loss of neutral HF from the molecule. The reaction mechanism for this process could be via a ring formation. The large intensity of m/z 165 could be due to a similar mechanism to produce the molecule shown in Fig. 5b. As expected, no chloride patterns were found for the m/z 156, 165 and 183 ions. The m/z 246–248 remain unidentified. The ions at 249 and 251 may be assigned to rhenium oxide from the filament.

At lower restrictor temperature (170°C) the mass spectrum was dominated by $[M]^{-\bullet}$ and $[M-HF]^{-\bullet}$ (Fig. 4b). Almost no chloride was observed in the mass spectra. The m/z 254 may originate from a $[M-HF-HCl]^{-\bullet}$ fragment. By comparing the RIC chromatograms of the above-mentioned m/z values, it was observed that the peak maxima of m/z 35/37, 156, 165 and 183 were slightly delayed compared to m/z 310, 290 and 254. A delay may be explained by the laminar flow through the heated zone and decomposition of diflubenzuron at the hot wall of the restrictor. This indicates that m/z 156, 165 and 183 originate from thermal decomposition occurring in the heated zone of the restrictor. Since m/z 290 and 254 have peak maxima identical to $M^{-\bullet}$, these fragment ions may be produced in the ion source. However, the possibility of these ions being formed by thermolysis must be considered.

The sensitivity was also found to be strongly dependent on restrictor temperature. Increased TIC (total ion current) peak heights with lower restrictor temperatures were found (Fig. 6). This would partly be explained if the degradation

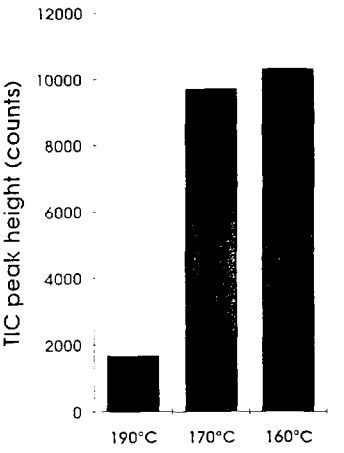


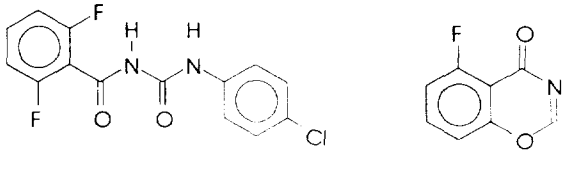
Fig. 6. TIC peak heights of diflubenzuron at three different restrictor temperatures.

products had a total cross-section for the EC processes which was lower than that of the analyte itself, thus producing a weaker total ion current. Another possibility is the effect of restrictor temperature on CO_2 cluster formation and production of fewer thermalized electrons.

The minimum detectable amount of diflubenzuron was estimated to be 100 pg and 3 pg injected onto the column in full-scan and SIM mode, respectively, using a signal-to-noise ratio of 3. This corresponds to detection limits of 1.0 μg/ml and 0.03 μg/ml.

Less thermal degradation and lower limit of detection (LOD) might be achieved by heating only the part of the restrictor where adiabatic expansion finds place. A restrictor heater with a more local heat zone would therefore be suitable. Such devices have been designed and used elsewhere [18,19].

a) b)



Diflubenzuron, MW: 310.7

Fig. 5. Diflubenzuron (a) and a possible structure for the main decomposition product at 190°C restrictor temperature (b).

4. Conclusions

Neat CO_2 can be used as the mobile phase for the chromatography of diflubenzuron on the highly deactivated Inertsil C_8 packing material. Ionization and detection by ECNI was obtained utilizing the mobile phase CO_2 as moderating gas. Generally lower limits of detection were observed using the modified ion source with

coaxial introduction used in this work compared to the earlier design [13].

The detection limits for diflubenzuron obtained in this work are not as low as those obtained by HPLC [3], mainly due to the lower acceptable sample introduction volume. However, the much better selectivity as well as the possibility of compound confirmation makes SFC–MS a promising complementary method for the determination of diflubenzuron in different samples.

The restrictor temperature was found to have a major effect on the signal intensity (TIC) and fragmentation pattern. To obtain molecular ions, a restrictor temperature of 160–170°C was found to be suitable for the detection of diflubenzuron using an ion source temperature of 150°C. The additional fragment ions observed at this restrictor temperature are advantageous for identification.

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