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A selective ^{19}F NMR spectroscopic method for determination of insecticide diflubenzuron in different media

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Abstract

A selective and rapid ^{19}F NMR spectroscopic analytical procedure for insect growth regulator diflubenzuron, a phenylurea insecticide, has been developed. Optimal NMR sensitivity based upon T_1 relaxation time was investigated for diflubenzuron and 4-fluorobenzoic acid as internal standard in dimethyl sulphoxide. The method was validated through spike and recovery for diflubenzuron from apple juice and its commercial sample (Dimilin) without any interference, separation steps and lengthy instrument time. The calibration curve was linear for diflubenzuron over the range of 25–1000 μg/ml, with detection limit ($S/N = 3$) of 6 μg/ml. The within- and between-day repeatability coefficients of variation were lower than 6 ($n = 3$) and 7 ($n = 5$), respectively. Recovery of diflubenzuron from spiked apple juice samples at two fortification levels were 96% and 92% with relative standard deviation of <10% ($n = 3$).

Keywords: ^{19}F NMR; Diflubenzuron; Apple juice; HPLC

1. Introduction

Diflubenzuron (1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea) (Scheme 1), the active ingredient of Dimilin, is a highly effective chemical insecticide used to control numerous forest and agricultural pests. It is a growth regulator, which acts by inhibiting chitin formation and disrupting normal growth and development processes in insects (Butler, Chrislip, Kondo, & Townsend, 1997). The incorrect use of this compound, such as other insecticide, may also result in the presence of its residues in agricultural products and, hence, in food commodities thereof, i.e., fruit juices and so on, thus compromising their safety.

Chromatographic methods have been employed for the determination of diflubenzuron in different matrices. Due to the thermal decomposition of diflubenzuron in the gas chromatography (GC) injector to form 2,6-difluorobenzamide, 4-chloroaniline and 4-chlorophenyl isocyanate (Wimmer, Smith, & Jones, 1991), direct determination by GC is not feasible. However, indirect GC methods based upon derivatization of hydrolysis products (Rabenort et al., 1978) or direct derivatization (Mensah, Lundanes, Greibrokk, & Holen, 1997) with subsequent electron-capture or mass spectrometric detection have used for quantification. The other methods available for direct quantization are based upon high-performance liquid chromatography (HPLC) with UV detection (Balinova, 1998; Valenzuela, Lorenzini, Redondo, & Font, 1999). This detection method loses sensitivity when extracts of complex environmental samples are analyzed because of interference caused by the UV-absorbing characteristics of many of the co-extracted compounds. Several derivatization methods have been used (Gil Garcya, Martynez Galera, Barranco Martynez, & Gisbert Gallego, 2006; Martinez-Galera, Lopez-Lopez, Gil-Garcia, Martinez-Vidal, & Parrilla Vazquez, 2001); however, all traditional methods for

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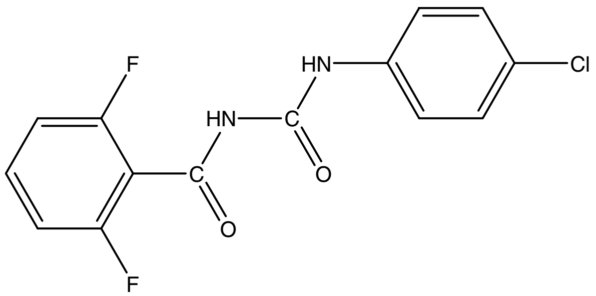

Scheme 1. Structure of diflubenzuron.

derivatization, extraction and clean-up steps are time- and solvent-consuming. Also, due to the long extraction process, degradation of the components can occur.

Recently, nuclear magnetic resonance (NMR) spectroscopy has been used for the analysis of drugs (Shamsipur, Shafiee-Dastgerdi, Talebpour, & Haghgoo, 2007; Talebpour, Haghgoo, & Shamsipur, 2004) as well as liquid food composition because it is capable of simultaneous detection of a great number of organic compounds and enables a more rapid and non-invasive characterization (Aman et al., 2005; Belton et al., 1998). Fluorine-19 nuclear magnetic resonance (^{19}F NMR) spectroscopy is a new selective method to study organofluorines and products resulting from their degradation (Green, Meharg, Till, Troke, & Nicholson, 1999; He et al., 2006). A wide range of chemical shift (~300 ppm) for the ^{19}F nucleus ensures good separation of signals in different environments, its 100% natural abundance and possessing a sensitivity which is 81% of a ^{1}H nucleus, makes ^{19}F NMR a reliable analytical tool for monitoring organofluorine compounds and their quantification, when an internal standard of known concentration is included without the need for separation and/or derivatization steps (Ellis, Martin, Muir, & Mabury, 2000).

With the aim to develop an efficient and practical screening method to investigate the residues of diflubenzuron in market foods (i.e., fruit juices), simple ^{19}F NMR techniques were implemented in this study. This method was successfully applied to monitoring of diflubenzuron in apple juice and a finished product (Dimilin). Experimental parameters for ^{19}F NMR analysis of the diflubenzuron were selected to optimize the NMR method with respect to accuracy, precision and analysis time. In order to validate suggested method, a comparative study on the determination of diflubenzuron was conducted by employing a HPLC method.

2. Experimental

2.1. Materials

Technical-grade diflubenzuron of 99% purity was obtained from Riedel de Haën (Seelze, Germany). Apple juice and a commercial grade diflubenzuron (Dimilin) samples were provided from local markets. The former sample was microfiltered through a 0.45 μm pore size filter. High purity 4-fluorobenzoic acid and deuterated dimethyl sulphoxide were purchased from Merck (Darmstadt, Germany). Methanol used in this study (Caledon Co., Georgetown, ON, Canada) was HPLC grade and LC-grade water was prepared by purifying in a Milli-Q water filtration system (Millipore, St. Quentin, France).

2.2. Instrumentation

2.2.1. ^{19}F NMR Instrument

All ^{19}F NMR spectra were recorded on a Bruker DRX 500 Avance (Ettlingen, Germany) (11.7 T) spectrometer operating at 470.59 MHz running XWIN-NMR 2.6 software. Five hundred microliters of each sample containing 100 μl deuterated dimethyl sulphoxide as an internal field-frequency lock was introduced into a 5 mm tube. The spectra were acquired using 90° pulses with 128–512 scans collected into 16 K computer data points over a spectral width of 61,162 Hz (130 ppm). The spectra were recorded at 300 K and chemical shifts were reported relative to trichlorofluoromethane ($CFCl_3$) at 0.00 ppm.

Fluorine nuclei were quantified using 4-fluorobenzoic acid as an internal standard. Measurement of T_1 by inversion recovery sequence gave values of 0.54 and 2.78 s for diflubenzuron and 4-fluorobenzoic acid, respectively. The relaxation delay of 10 s was thus sufficient to achieve a complete relaxation fluorine nucleus.

To attain reliable results the phasing and the baseline correction over the entire spectral range are critical, so these processes were carried out manually. In all instances, the baseline was additionally corrected over the integrated regions. Areas of the peak were measured by electronic integration of expanded regions around selected resonances using an integral limit of ±20 Hz around the corresponding signals.

2.2.2. HPLC apparatus and chromatographic conditions

A Kontron Model 420 pump, valve injector Rheodyne (Rohnert Park, CA, USA) equipped with a 20-μl loop and a Kontron Model 432 detector set to 254 nm was employed with a Chromgate software (Knauer, Germany) for acquisition system. Chromatography was carried out on a 5 μm C_{18} (Knauer, Berlin, Germany) column (250 mm × 4.6 mm ID). The mobile phase was methanol–water (80–20, v/v) delivered at a flow-rate of 0.7 ml/min. All solvents and samples were filtered through a 0.22 μm Millipore membrane filters type GVWP and mobile phases were degassed by a Selecta Ultrasounds System (Selecta, Barcelona, Spain) before use.

2.3. Sample preparation

Standard stock solution of diflubenzuron and 4-fluorobenzoic acid as internal standards were obtained by dissolving 10 mg of each compounds in 5 ml of dimethyl sulphoxide separately. Ten working standard solutions were prepared at concentrations from 25 to 1000 μg/ml and 200 to 600 μg/ml of diflubenzuron and 4-fluorobenzoic acid, respectively, by appropriate mixing and diluting of the stock solutions. All solutions were kept in the dark at 4 °C and all experiments were carried out in triplicate.

A set of calibration solutions for HPLC–UV analysis at concentrations from 1 to 800 μg/ml ($n = 10$) of diflubenzuron were prepared by appropriate dilution of the stock solution (1000 μg/ml). The concentration of diflubenzuron was determined by comparing the peak areas in the sample with those found in the calibration standard solutions. Each sample or standard was analyzed in triplicate.

2.4. Method validation

The quantification of diflubenzuron was performed using the internal standard method with integration of the fluorine signal of diflubenzuron related to fluorine signal of internal standard in each calibration solution. Fluorine peak-area ratios were plotted against the corresponding diflubenzuron concentration and a linear regression variance analysis was performed to determine linearity and correlation coefficients. The detection limit (DL) was calculated by using a signal-to-noise (S/N) ratio of 3.

Accuracy was assessed by determining the concentration of diflubenzuron in spiked samples (343, 560 and 776 μg/ml of diflubenzuron; $n = 3$ for each case) relative to the known concentration added. Also, the obtained results by ^{19}F NMR method were compared with those from a HPLC method. Precision was determined utilizing the coefficient of variation (CV, %) of the within-day ($n = 3$) and between-day ($n = 5$).

Recovery studies were carried out by spiking fresh samples (300 ml) of apple juices with two levels of diflubenzuron (6.0 and 60 μg/ml) and 4-fluorobenzoic acid (3.3 and 33 μg/ml). The solvent was allowed to evaporate and concentrate. The 2.15 g of its residue was redissolved in 4.0 ml dimethyl sulphoxide with the aid of an ultrasonic bath. Four hundred microliters of this solution were transformed to an NMR tube and mixed with 100 μl of deuterated dimethyl sulphoxide. ^{19}F NMR spectrum of this and untreated sample of apple juice were carried out in triplicate. In order to the determination of diflubenzuron in its commercial sample, a solution of this sample was prepared by dissolving of 12.5 mg of commercial sample of diflubenzuron in 25 ml of dimethyl sulphoxide. This solution was analyzed by ^{19}F NMR without any sample preparations.

3. Results and discussion

3.1. ^{1}H and ^{19}F NMR spectra of diflubenzuron

Fig. 1a shows the ^{1}H NMR spectrum of diflubenzuron in dimethyl sulphoxide as a suitable solvent not only for good solubility of diflubenzuron but also for its stability during the analysis. The three multiple peaks at 7.23–7.26, 7.38–7.40 and 7.60–7.64 ppm ranges were assigned to aromatic protons near nitrogen, chlorine and fluorine atoms, respectively, and two peaks appearing above 10 ppm corresponded to protons jointed to nitrogen atoms. It should be noted that, in ^{1}H NMR spectrum, one sharp singlet peak was observed at 2.49 ppm, which related to dimethyl sulphoxide.

Fig. 1b shows the ^{19}F NMR spectrum of diflubenzuron in dimethyl sulphoxide, possessing a sharp singlet ^{19}F signal for the two fluorine atoms at −113.44 ppm. The comparison of ^{1}H and ^{19}F NMR spectra of diflubenzuron shows that the wide range of fluorine chemical shifts in ^{19}F NMR spectroscopy and free interference due to the presence of other compounds in different media are advantages of ^{19}F NMR method for the monitoring of this compound. Also, this method introduces a selective and simple method for the monitoring of diflubenzuron in complex media without any separation and time-consuming sample preparations. Thus, analysis of diflubenzuron in fruit juice was performed with ^{19}F NMR spectroscopy.

3.2. Quantitative analysis

3.2.1. Optimization of NMR acquisition conditions

To obtain a high accuracy in quantitative analysis the elapsed time between the successive acquisitions of the spectra must be 3–5-fold the maximum T_1-value for maximal errors of 5% and 1%, respectively (Cookson & Smith, 1982), and these values were measured using the inversion recovery pulse sequence for frequencies offset centered on the signals of diflubenzuron and 4-fluorobenzoic acid. The T_1 values for signals of diflubenzuron and 4-fluorobenzoic acid were obtained at 0.54 and 2.78 s, respectively, therefore by using a delay time of 10 s, the total relaxation of the fluorines involved in the quantitative measure has been achieved.

Also, other factors which influence absolute intensities, such as variable sample volumes, spectrometer performance and B_1 inhomogeneity over the sample will be compensated for by the use of relative quantification. It is usually for this reason that the quantification in NMR spectroscopy is performed with the aid of an internal standard. Selection of the internal standard was dictated by high purity, chemical inertness, easy solubility and good resolution from the analyte signal. In this work, 4-fluorobenzoic acid was selected as a suitable internal standard, possessing a sharp singlet ^{19}F signal at −107.36 ppm well resolved from ^{19}F signal of diflubenzuron and the area from diflubenzuron signal was normalized with respect to that of 4-fluorobenzoic acid for each spectrum.

3.2.2. Method validation

3.2.2.1. Linearity and detection limit. To determine the linear range for diflubenzuron, a series of working standard solutions ($n = 12$) containing diflubenzuron and 4-fluorobenzoic acid over the range 25–1000 and 200–600 μg/ml,

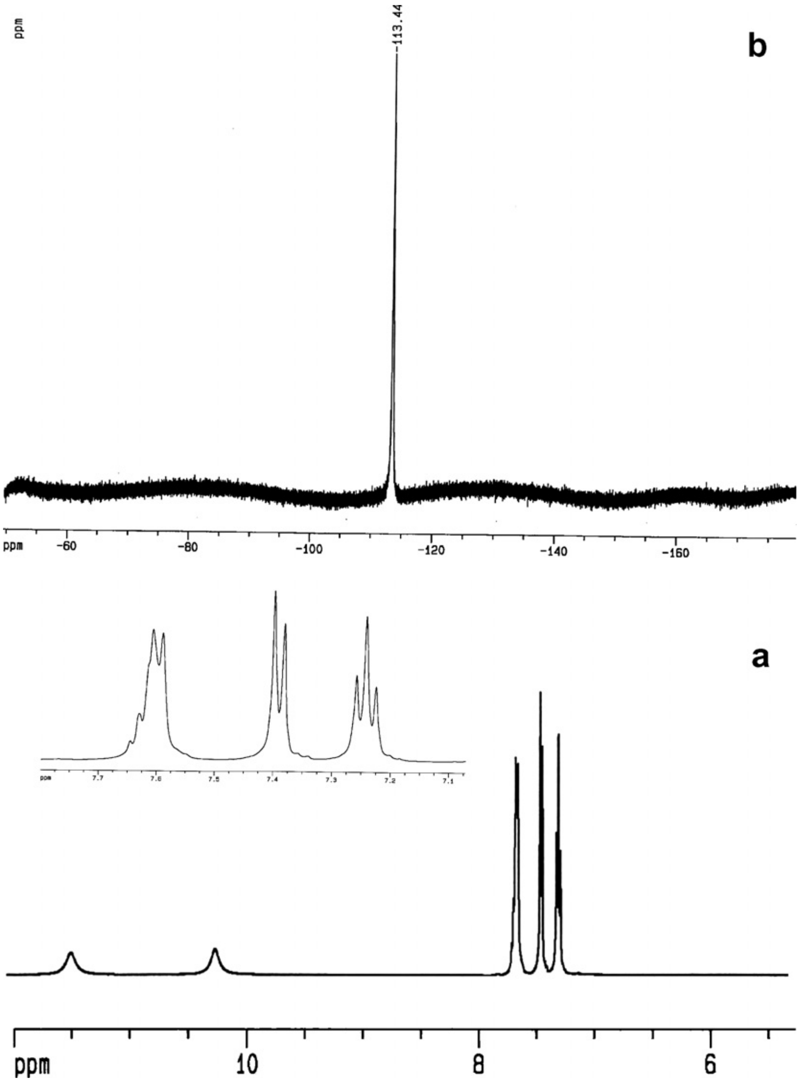

Fig. 1. (a) ^{1}H NMR spectrum of diflubenzuron in dimethyl sulphoxide and (b) ^{19}F NMR spectrum of diflubenzuron in dimethyl sulphoxide.

respectively, were prepared. The normalized peak-area of diflubenzuron relative to internal standard as a function of the concentration of diflubenzuron in each standard solution allowed the creation of a calibration curve. The calibration data are summarized in Table 1. Correlation coefficient was >0.9959, which indicates a good linearity within the concentration range studied. From this result and assuming a minimum usable sample volume of 500 µl and 128 scans, the detection limit (S/N = 3) under the optimized experimental conditions was approximately 6 µg/ml for diflubenzuron.

3.2.2.2. Accuracy and precision. The results obtained for diflubenzuran were accurate and precise as summarized in Table 2. The accuracy was demonstrated as the concentration of diflubenzuron measured in the three samples relative to the corresponding known concentrations (343, 560 and 776 µg/ml of diflubenzuron; $n = 3$ for each case) was 91–105%. The precision of the proposed method, as determined by the coefficient of variation of the within-day ($n = 3$) and between-day ($n = 5$) of above-mentioned samples was consistently within 4–7%. The accuracy and precision were quite acceptable.

Table 1
Linearity and detection limit for analyses of diflubenzuron

	Linear range (µg/ml)	Slop ± SD[a]	Intercept ± SD	Correlation coefficient (*R*)	*F*-value	DL[b] (µg/ml)
Diflubenzuron	25–1000	0.0081 ± 0.0091	0.0826 ± 0.0091	1433	6	

[a] Standard deviation.
[b] Detection limit.

Table 2
Accuracy and precision of ^{19}F NMR method

Spiked concentration of diflubenzuron (µg/ml)	Measured concentration (µg/ml)[a]		Accuracy (recovery, %)		Precision CV[b] (%)	
	Within-day ($n = 3$)	Between-day ($n = 5$)	Within-day ($n = 3$)	Between-day ($n = 5$)	Within-day ($n = 3$)	Between-day ($n = 5$)
343	357 ± 15	349 ± 19	104	102	4	5
560	523 ± 30	510 ± 35	93	91	6	7
776	821 ± 40	817 ± 46	103	105	5	6

[a] Mean ± S.D.
[b] Coefficient of variation.

3.3. Comparison ^{19}F NMR and HPLC results of diflubenzuron

Characteristics of calibration curve for analysis of diflubenzuron were obtained using a HPLC method. Ten working standard diflubenzuron solutions of different concentrations (1–800 µg/ml) were analyzed and the detector response (area) was plotted against concentration (C). Correlation was calculated by least square regression. The linear equation and correlation of coefficient was found area = 52280 C – 123516 and 0.9993, respectively. The accuracy and precision of ^{19}F NMR was compared to HPLC method and summarized in Table 3. As can be observed, lower recoveries were achieved by HPLC method. Although limit of the detection of HPLC (0.1 µg/ml) is better than ^{19}F NMR (6 µg/ml), selectivity and simplicity of this method are very higher than chromatographic methods in complex media of food samples.

3.4. Applications

Apple juice was selected as a model of food sample for monitoring diflubenzuron. The recovery of diflubenzuron was carried out using an untreated apple juice sample fortified at two concentration levels (6 and 60 µg/ml equivalent to 67 and 670 µg/ml in the final extract) – one near the limit of detection of the method and the other – 10 times higher. ^{19}F NMR spectrum of this sample is shown in Fig. 2. The peak at −113.50 ppm indicates the presence of diflubenzuron in apple juice without any interference.

The recovery of diflubenzuron from apple juice was 96 ± 10% for 6 µg/ml and 92 ± 6% for 60 µg/ml levels. These results indicate good accuracy and precision for the method compared with the currently available methods which involve several additional steps.

Table 3
Comparison of ^{19}F NMR and HPLC methods for analysis of diflubenzuron

Spiked concentration of diflubenzuron (µ/ml)	Recovery (%)		CV (%)[a]	
	^{19}F NMR	HPLC	^{19}F NMR	HPLC
144	95	89	6	3
384	103	89	6	3
560	93	84	5	3

[a] Coefficient of variation.

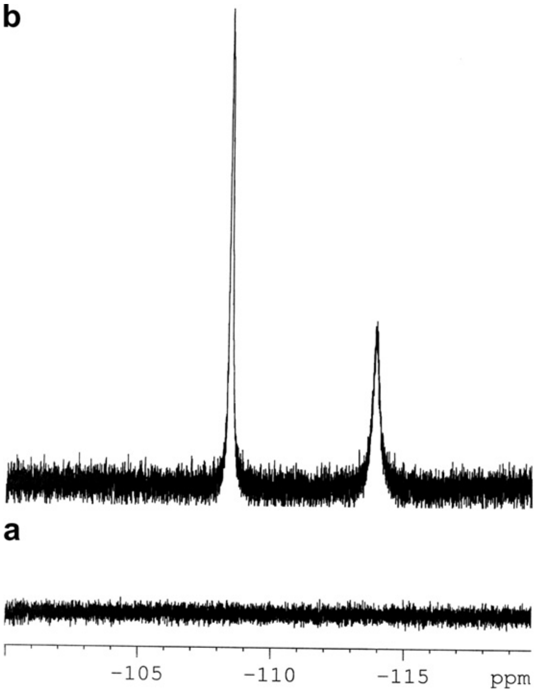

Fig. 2. ^{19}F NMR spectrum of (a) untreated apple juice and (b) spiked diflubenzuron and 4-fluorobenzoic acid to apple juice at 6.0 and 1.0 µg/ml, respectively.

The amount of diflubenzuron in finished product sample (Dimilin) was determined by ^{19}F NMR spectroscopic method. The ^{19}F NMR spectrum of this sample was recorded without any sample preparation and the sample was only dissolved in dimethyl sulphoxide. The recovery and standard deviation of diflubenzuron in commercial sample were obtained at 98% and 4, respectively.

The percent recoveries for spike and recovery experiments indicated that the accuracy of the method was acceptable, and the percent CV-value for apple juice and commercial sample performed in triplicate indicated good precision for the ^{19}F NMR methodology.

3.5. Conclusions

Selectivity of NMR spectroscopy methods permits the identification of different compounds in various media. If this ability accompanies with quantitative accuracy, a practical method can be presented. The present ^{19}F NMR spectroscopy method was successfully employed for monitoring of diflubenzuron pesticide in apple juice and its finished product. This method could be potentially extended to other matrices and offers advantages in terms of its speed and simplicity for sample analyses. A further gain is the possibility of the simultaneous determination of other fluorinated components in the same sample, in particular degradation products of diflubenzuron.

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