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Determination of clothianidin in food products by using an automated system with photochemically induced fluorescence detection

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ABSTRACT

A flow-through system based on the integration of solid-phase spectroscopic detection implemented with photochemically induced fluorescence (PIF) is proposed for the determination of clothianidin (a non-fluorescent neonicotinoid insecticide) through a multicommutated method. The pesticide is injected into the carrier stream ($0.015\ mol\ L^{-1} C_2H_4O_2/NaC_2H_3O_2$, pH = 5.0) and flows towards a homemade photoreactor, which consists of a PTFE tubing loosely coiled around a low-pressure mercury lamp (15W). After the photochemical reaction of clothianidin, the generated fluorescent photoproduct is transported to a flow cell packed with Sephadex-SP C-25 where it is retained and monitored (λ_{ex} = 357 nm/λ_{em} = 418 nm). The method presents a detection limit of $1.5\ ng\ mL^{-1}$, a sample throughput of $23\ h^{-1}$ and inter-day relative standard deviation lower than 3%. The described system has been satisfactorily applied to the determination of clothianidin in samples of drinking water, rice and honey. Taking into account that the maximum residue limit specified in the Codex Alimentarius Commission for rice grains is $0.5\ mg\ kg^{-1}$, recovery experiments have been carried out for clothianidin concentrations in the 0.3-10.0 $mg\ kg^{-1}$ range.

1. Introduction

Neonicotinoids are a class of insecticides deriving from the nicotine moiety. Their use has increased considerably in the last few decades, and they represent one of the fastest growing types of insecticides. Both food quality and safety are affected by their application to crops at various stages of cultivation and during the post-harvest storage, thus causing damage to the final link in the food chain, namely, the consumer.

Clothianidin (Fig. 1), a member of this family, has been widely used for long-term control of a wide variety of pests (Tomizawa and Casida, 2005; Uneme, 2011); it is also used in seed dressings and added to the soil (Sánchez-Bayo et al., 2013). Clothianidin is receiving increased scrutiny since it has been implicated in adversely affecting pollinators and linked to colony collapse disorder in insects such as bees. Therefore, studying its use on different types of food crops is gaining much interest.

The Codex Alimentarius Commission (CAC) has stipulated that the Maximum Residue Limits (MRLs) of clothianidin remain within the range 0.02–2.0 $mg\ kg^{-1}$, depending on type of food (cereals, vegetables or fruits) (2015). For this reason, it is necessary to detect clothianidin residues by sensitive and rapid methods in order to protect non-target organisms and environment from damage. Specifically, the European Commission banned its use in December 2013, finding that this chemical is harmful to bees. When honeybees come into contact with this compound, it may be taken along with the bees into the beehive, and their residues may then be found in bee products such as honey (Jovanov et al., 2013). In April 2014, the Reference Laboratory of the European Union for Health of Bees published the results of the first program to monitor the depopulation of hives in 17 European countries. The data show a highly variable rate of winter mortality between countries, ranging from 3.5 to 33.6%. In general, the situation is milder in the Mediterranean countries than in northern Europe. Restricting the use of clothianidin in agriculture will be reviewed within a maximum term of two years.

Currently available methods for the determination of clothianidin are based mainly on high performance liquid chromatography (HPLC) (Chen et al., 2013, 2005; Hou et al., 2011; Kim et al., 2012; Vichapong et al., 2013, 2015) and gas chromatography (GC) (Li et al., 2012). Although these methods are very sensitive and precise, they are not suitable for high-throughput and rapid screening of large numbers of samples. Thus there is much interest in exploiting less-expensive yet reliable methods for the determination of this compound. Among these methods, fluorescence

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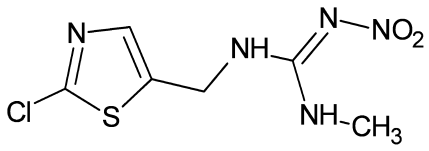

Fig. 1. Structure of clothianidin.

methods are generally rapid and reliable, but their application rather limited by the fact that neonicotinoids (including clothianidin) are not naturally fluorescent, and must therefore be converted into fluorescent species.

An alternative method, so-called photo-induced fluorescence (PIF), is based on the photochemical transformation of a non-fluorescent or weakly fluorescent analyte into strongly fluorescent photoproduct(s) by using UV irradiation (Icardo and Calatayud, 2008; Mbaye et al., 2009). Sensitivity and selectivity can be increased by coupling PIF and solid-phase spectrometry (SPS) (Molina-García et al., 2011b). Beads of an active surface (solid support) can be placed into an appropriate flow-cell, thus obtaining a continuous sensing device that allows on-line species monitoring (Ayora Cañada et al., 2002; Ruiz-Medina et al., 2001). The solid support has to be regenerated after measurements to allow the sensing system to be reused. In these systems, called flow-through sensors, separation and preconcentration steps occur simultaneously with the detection step (Llorent-Martínez et al., 2013; Ruedas Rama et al., 2004).

Automation in the method can be achieved in various ways. Garcia et al. (1996) were the first to determine pesticides by combining the PIF technique with flow-injection analysis (FIA). As alternative to FIA, new and modern flow methodologies such as multicommutated approaches can be used (Feres et al., 2008; Llorent-Martínez et al., 2011). Multicommutated flow injection analysis (MCFIA) (Cerda and Pons, 2006; Chailapakul et al., 2006; Jiménez-López et al., 2014) has been selected as automatic methodology due to its low-cost components, low consumption of reagents, high repeatability and sample throughput, robustness and simplicity of the system, which was designed to make use of three-way solenoid valves automatically controlled by a computer with homemade software.

The objective of this paper is twofold: (i) to develop a sensitive and selective multicommutated flow method for detecting trace clothianidin residues by measuring the fluorescence of one of its photodegradation products generated, so providing an alternative to the existing chromatographic methods; and (ii) to validate the method using different types of samples from the environment and found in the agro-food sector: water, cereals and honey. QuEChERS (quick, easy, cheap, effective, rugged and safe) method (Anastassiades et al., 2003) was applied as sample pre-treatment to extract and separate the analyte from some interfering substances present in the matrix. To the best of our knowledge, a fluorimetric system is being proposed for the first time for the analysis of clothianidin in these products.

2. Materials and methods

2.1. Instrumentation

A four-channel Gilson Minipuls-3 (Villiers Le Bel, France) peristaltic pump with rate selector and methanol-resistant pump tubes, type Solvaflex (Elkay Products, Shrewsbury, MA, USA) were used. An electronic interface based on ULN 2803 integrated circuit was employed to generate the electric potential (12 V) and current (100 mA) required to control the four 161T031 NResearch three-way solenoid valves (Neptune Research, MA, USA). The software for controlling the system was written in Java. Flow lines of 0.8 mm internal diameter PTFE tubing and methacrylate connections were also used.

A homemade continuous photochemical reactor was constructed by coiling a PTFE tubing (180 cm) around a low-pressure mercury lamp (15 W, 254 nm). This reactor has already been used in other systems proposed by the authors (Jiménez-López et al., 2016; Molina-García et al., 2011a; Molina-García et al., 2011c). It was placed inside an aluminum box to permit the maximum reflectance of UV light. Since the aluminum foil allowed heat dissipation, no cooling device was needed, and all the experiments were carried out at room temperature.

A Varian Cary-Eclipse spectrofluorimeter (Varian, Mulgrave, Melbourne, VIC, Australia) was used for recording spectra and making fluorescence measurements. It was controlled by a microprocessor fitted with the Cary Eclipse software package. The instrumental variables established were as follows: 357 and 418 nm for excitation and emission wavelengths, respectively; 5 and 10 nm for excitation and emission slits, respectively; and 750 V for photomultiplier tube voltage. A Hellma flow cell 176.752-QS (25 μL of inner volume and a light path length of 1.5 mm) was used inside the spectrofluorimeter. The cell was filled with Sephadex SP C-25 solid phase microbeads, and was blocked at the outlet with glass wool to prevent displacement of the support particles. The level of the resin packed in the flow cell had to be carefully selected in order to ensure that the upper part of the resin fell precisely in the light path. In this way, the best sensitivity was obtained. Before starting the measuring process, the carrier solution was passed through the sensing zone for 5 min to condition it.

A Selecta Ultrasons ultrasonic bath (Barcelona, Spain), a Crison Model 2012 pH-meter with a glass/saturated calomel combination electrode (Crison, Barcelona, Spain), and a centrifuge (Selecta, Barcelona, Spain) were also used.

2.2. Reagents and solutions

Clothianidin (≥99.9%) was purchased from Fluka (Sigma-Aldrich, Madrid, Spain). Stock solution of 100 μg mL^{-1} clothianidin was prepared in Mili-Q water (Sigma, Madrid, Spain). It was stable for at least 1 month when stored in the refrigerator at +4 °C. Working solutions were prepared daily by suitable dilution with Milli-Q water.

Acetonitrile (C_2H_3N), acetic acid ($C_2H_4O_2$), hydrochloric acid (HCl), anhydrous sodium acetate ($NaC_2H_3O_2$), sodium hydroxide (NaOH), and anhydrous magnesium sulfate ($MgSO_4$) were obtained from Sigma. Sodium dodecyl sulfate (SDS), Triton X-100 and purified and calcined siliceous earth were purchased from Panreac (Barcelona, Spain). Formic acid (CH_2O_2), sodium formate ($NaCHO_2$), citric acid ($C_6H_8O_7$), sodium citrate ($C_6H_7NaO_7$) and succinic acid ($C_4H_6O_4$) were purchased from Sigma (Madrid, Spain). All of them were analytical reagent grade.

The carrier stream consisted of 0.015 mol L^{-1} $C_2H_4O_2/NaC_2H_3O_2$ (pH 5.0) buffer solution. Stock solution (0.2 mol L^{-1}) was prepared by dissolving 1.8915 g of $NaC_2H_3O_2$ with deionized water and adjusting the pH to 5.0 with the appropriate amount of 0.1 mol L^{-1} $C_2H_4O_2$ to 100 mL of final volume. The eluent used to fully regenerate the solid support in the analysis of real samples was 0.005 mol L^{-1} NaOH solution.

Sephadex-QAE A-25, Sephadex-CM C-25, and Sephadex-SP C-25 resins, all of them 40–120 μm average particle size, were bought from Sigma. C_{18} bonded-phase silica gel beads, 55–105 μm average particle size, were obtained from Waters (Milford, MA, USA). Chelex-100 in sodium form, 200–400 mesh, was purchased from Fluka (Buchs, Switzerland).