Journal of Chromatography A, 1394 (2015) 1–8

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Dispersive liquid–liquid microextraction for the determination of new generation pesticides in soils by liquid chromatography and tandem mass spectrometry☆

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ARTICLE INFO

Article history:
Received 12 November 2014
Received in revised form 20 February 2015
Accepted 16 March 2015
Available online 23 March 2015

Keywords:
Anthranilic diamides
Dispersive liquid–liquid microextraction
Liquid chromatography-tandem mass spectrometry
Pesticides
Soils
Spirocyclic tetronic acid derivatives

ABSTRACT

A sensitive method for the determination of five new generation pesticides (chlorantraniliprole, spirotetramat, spiromesifen, spirodiclofen and flubendiamide) in soil samples has been developed using liquid chromatography and tandem mass spectrometry (LC-MS^2) with a triple-quadrupole in the multiple reaction monitoring mode. The target analytes are released from the solid matrix by single-phase extraction in acetonitrile (SLE), this organic phase being used as dispersant solvent in the dispersive liquid–liquid microextraction (DLLME) step. The different parameters affecting the extraction efficiency in DLLME were carefully studied, being 1.5 mL of acetonitrile extract (disperser solvent), 125 μL carbon tetrachloride (extraction solvent) and 10 mL aqueous solution, the selected conditions. The enriched organic phase was evaporated, reconstituted with 50 μL acetonitrile and injected into a liquid chromatograph with a mobile phase composed of acetonitrile and 0.1% (v/v) formic acid under gradient elution and a C_8 stationary phase. Detection limits in the 0.0015–0.0090 ng g^{-1} range were obtained. Insecticide concentrations ranging from 0.03 to 197 ng g^{-1}, depending on the compound, were found in the soil samples analysed. The recovery values obtained by SLE-DLLME-LC-ESI-MS^2 for three spiked soils at three concentration levels varied between 87 and 114%, with RSDs of between 5.5 and 14%.

1. Introduction

Spirocyclic tetronic/tetramic acid (ketoenol) derivatives (spirodiclofen, spiromesifen and spirotetramat) and anthranilic/phthalic (chlorantraniliprole and flubendiamide) diamides are two of the most recently developed classes of acaricide/insecticide for the control of a wide spectrum of sucking insects in numerous agricultural crops [1–4]. Spirodiclofen, spiromesifen and spirotetramat interfere with lipid biosynthesis and are thought to act as inhibitors of acetyl-coenzyme A carboxylase (ACCase) [5–7]. Chlorantraniliprole and flubendiamide both act as activators of insect ryanodine receptors by stimulating the release and depletion of intracellular calcium stores, causing the rapid cessation of feeding, lethargy, regurgitation, muscle paralysis and, ultimately, the death of sensitive species [8,9]. In general, the use of these insecticides provides unquestionable benefits in terms of increased production and quality. However, a certain proportion of the product used may reach the soil, constituting a risk to the environment and society in general.

Because of the complexity and diversity of soil samples, the different physicochemical properties of pesticides and the low concentrations expected for these pollutants in these matrices, sample preparation is one of the most important steps for the determination of different pesticides in soil media. For this reason, many methods have been used to extract pesticide residues from soil samples, including Soxhlet extraction [10], solid-phase extraction (SPE) [11], supercritical fluid extraction (SFE) [12], solid–liquid extraction (SLE) [13], ultrasound assisted extraction (UAE) [14,15], microwave assisted extraction (MAE) [16], pressurized liquid extraction (PLE) [17], solid-phase microextraction (SPME) [18] and the QuEChERS method [19,20].

☆ Presented at the 30th International Symposium on Chromatography (ISC 2014), Salzburg, Austria, 14–18 September 2014.

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http://dx.doi.org/10.1016/j.chroma.2015.03.032
0021-9673/

In recent years, miniaturized sample preparation methods combined with chromatographic techniques have led to the development of green analysis methods, which have permitted with high extraction rate, great efficiency, high selectivity and low costs [21]. Dispersive liquid–liquid microextraction (DLLME) is a very simple and successful miniaturized technique which consists of the generation of micro-drops of the acceptor phase inside the aqueous phase by using a dispersant solvent, thus increasing the contact surface and, consequently, making extraction almost instantaneous [22–24], with high enrichment factors attained in most applications. This technique has been used for the preconcentration of a wide number of compounds in different samples, including soils [25,26], and its possibilities have greatly increased owing to several modifications, such as the use of extractant solvents lighter than water and the derivatization of the analytes, among many others.

The literature mentions a very reduced number of chromatographic methods for spirocyclic tetronic/tetramic acid derivatives and anthranilic/phthalic diamides insecticides, having been determined in soils [27–29], soluble concentrate formulations [30], tobacco [31] and fruits and vegetables [32–40]. In soil matrices, only spirotetramat [27,29] and chlorantraniliprole [28] have been analysed, in some cases by applying classic solid–liquid extraction methods involving high amounts of organic solvent. The determination of chlorantraniliprole in soils [28] also included an extract purification step by means of SPE. To the best of our knowledge, only one previous work has dealt with the determination of one of the above pesticides in fruit juices, spirodiclofen, involving a microextraction procedure using an ionic liquid [37]. The combination of DLLME and chromatographic techniques for the analysis of these pesticides in soils has not been reported. The aim of this work was to develop a simple, sensitive and new method for the determination of five new pesticides (spirodiclofen, spiromesifen, spirotetramat, chlorantraniliprole and flubendiamide) in different soil matrices using SLE combined with the environmentally friendly DLLME technique and liquid chromatography with tandem-mass spectrometry (LC-MS^2), using electrospray ionization (ESI) and triple quadrupole in multiple reaction monitoring (MRM) mode.

2. Experimental

2.1. Chemicals and reagents

Residue analysis grade acetonitrile was purchased from Scharlau (Barcelona, Spain). Deionized water was obtained from a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA). Formic acid (98% purity) and sodium chloride were ordered from Fluka-Sigma–Aldrich (Steinheim, Germany). Pesticide standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany) with a purity >98%: chlorantraniliprole (3-bromo-4′-chloro-1-(3-chloro-2-pyridyl)-2′-methyl-6′-(methylcarbamoyl)pyrazole-5-carboxanilide), spirotetramat (*cis*-3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl ethyl carbonate, *cis*-4-(ethoxycarbonyloxy)-8-methoxy-3-(2,5-xylyl)-1-azaspiro [4.5]dec-3-en-2-one), spiromesifen (3-mesityl-2-oxo-1-oxaspiro [4.4]non-3-en-4-yl) 3,3-dimethylbutyrate), flubendiamide (N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-{2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl}-1,2-benzenedicarboxamide) and spirodiclofen (3-(2,4-dichlorophenyl)-2-oxo-1-oxaspiro[4.5]dec-3-en-4-yl) 2,2-dimethylbutyrate). The chemical structures of the five insecticides are shown in Fig. 1. Stock solutions (1000 μg mL^{-1}) of each compound were prepared by dissolving 0.025 g of each in 25 mL of acetonitrile. Individual standard solutions for the optimization and standard mix solution (10 μg mL^{-1}) for the calibration were prepared in acetonitrile from the stock standards and were kept at −18 °C before use.

2.2. Apparatus

For the LC analysis, an Agilent 1100 HPLC system with a binary pump was used. This was equipped with a reversed phase C8 analytical column of 150 mm × 4.6 mm and 5 μm particle size (Zorbax Eclipse XDB-C8). The mobile phases A and B were acetonitrile and 0.1% (v/v) formic acid, respectively, operating under gradient elution. The optimized programme consisted of an isocratic step with 10:90 A:B mixture for 5 min, then a linear gradient to 100% A in 30 min, which was held for 5 min. Finally, the initial conditions were re-established in 1 min and held for 2.5 min. The flow-rate was held constant (0.6 mL min^{-1}) during the whole process. Injection (20 μL) was performed using an autosampler and vials of 2 mL capacity provided with 250 μL micro-inserts with polymeric feet.

For the mass spectrometric analysis, an Agilent G6410A triple quadrupole mass spectrometer was used. The ESI source was operated in positive and negative ion mode, using the following operation parameters: capillary voltage, 4000 V; nebulizer pressure, 40 psi; drying gas flow, 9 L min^{-1} and drying gas temperature, 350 °C. Mass spectra were recorded in the range of *m/z* from 50 to 1000 amu. Nitrogen served as the nebulizer and collision gas. Agilent Mass Hunter Data Acquisition (Qualitative Analysis and Quantitative Analysis) software was used in the method development and for data acquisition.

A preliminary study of the optimal selected reaction monitoring (SRM) transitions for every compound was carried out by directly infusing into the ion source every individual analyte at a concentration of 10 μg mL^{-1}; various fragmentor voltages and collision energies were applied to the compounds under study (Table 1). The identification procedure for insecticide residues in soil was carried out using the retention time and two SRM transitions, involving the formation of product ions with higher *m/z* value, being most sensitive transition used as quantifier and both as qualifier peaks for the confirmatory analysis.

A rotatory shaker Heidolph Reax 2 (Schwabach, Germany) and an EBA 20 (Hettich, Tuttlingen, Germany) centrifuge were used to treat the samples.

2.3. Samples and analytical procedure

Soil samples were taken from greenhouses, where the pesticides under study had been used throughout the crop cycle cultivation, in Campo de Cartagena, Murcia (south-eastern, Spain). Soil samples were collected from the surface (top 20 cm), air-dried, and passed through a 2 mm sieve. A total of ten samples were analysed. For the optimization of the sample procedure three soil samples were used (soil samples A, B and C). These were characterized as 29.1, 32.9 and 31.7% clay, 33.4, 30.2 and 23% silt, 37.5, 36.9 and 45.3% sand, 0.22, 0.51 and 0.17% total nitrogen, 0.9, 1.8 and 1.8% total organic carbon, 7.9, 7.3 and 7.7 pH (H_2O 1:1), and 1300, 7300 and 6400 μS cm^{-1} electric conductivity, respectively, measured at 25 °C.

Extraction of the analytes from the solid matrices followed the method of Fenoll et al. [15] with light modifications: 6 mL of a acetonitrile:water (1:1) were added to 2 g soil previously weighed in a centrifuge tube. The mixture was shaken by means of a rotator agitator for 3 min at maximum power, followed by a salting-out step involving the addition of 0.5 g sodium chloride. Next, the tube was manually shaken and centrifuged for 5 min at 3000 rpm. 1.5 mL of ACN was recovered to be used as dispersant solvent in the subsequent DLLME step. 125 μL of carbon tetrachloride was added to 1.5 mL acetonitrile and the mixture was rapidly injected by means of a syringe into 10 mL water placed in a conical bottomed glass centrifuge tube. A cloudy solution consisting of very fine droplets of CCl_4 dispersed through the aqueous phase was obtained,