Food Chemistry 130 (2012) 1090–1097

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Analytical Methods

Optimisation of a solid-phase microextraction/HPLC/Diode Array method for multiple pesticide screening in lettuce

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ARTICLE INFO

Article history:
Received 16 December 2010
Received in revised form 4 May 2011
Accepted 31 July 2011
Available online 16 August 2011

Keywords:
Pesticides
Lettuce
Quantitative methods
Response surface methodology

ABSTRACT

A new method was developed for the determination of 10 pesticides widely used in lettuce production (acetamiprid, azoxystrobin, cyprodinil, fenhexamid, fludioxonil, folpet, iprodione, metalaxyl, pirimicarb, and tolyfluanid) using solid-phase microextraction (SPME) and liquid chromatography (HPLC) with diode-array detection (DAD). The extraction performance of four different SPME coatings, polydimethylsiloxane (PDMS), PDMS/divinylbenzene (PDMS/DVB), carbowax/templated resin (CW/TPR), and polyacrylate (PA) was evaluated using an interface SPME–HPLC. CW/TPR fibre was selected as the most appropriate for the extraction of majority of these pesticides. Three variables (pH, NaCl% and extraction time) were considered key factors in the optimisation process. Interactions between these analytical factors and their optimal levels were investigated by response surface methodology based on central composite design. The method allowed the determination of azoxystrobin, cyprodinil, fenehexamid, fludioxonil, folpet, iprodione, and tolyfluanid in lettuce at concentrations between 0.8 and 25.6 mg/kg, i.e., bellow the maximum residues levels allowed for those compounds in lettuce. Lettuce samples that suffered pesticide treatments with folpet and fenehexamid were analysed during days to harvest to study the dissipation behaviour of the pesticides used. Concentration of folpet was 92.8; 53.4; 22.9; 17.9; 7.45; 1.85 mg/kg and concentration of fenhexamid was 158.1; 76.2; 31.0; 27.3; 7.24; 0.87 mg/kg, respectively for t_0, t_1, t_3, t_6, t_7, t_9 days, and not detected at t_{14} days for the two pesticides.

1. Introduction

Pesticides are potentially harmful to the environment and consequently to human beings not only by direct contact but also through the consumption of pesticide contaminated food and water. The European Community established maximum residue levels (MRLs) based on the assumption that good agricultural practise is applied at the use of pesticides in farming (Regulation (EC) No. 396/2005).

Governmental legislation demands an increasing number of pesticides to be included in the monitoring programmes (Directive 2009/128/EC). This forces the laboratories to look for effective methods capable of screening an increasing number of pesticides. Thus, the application of multiresidue methods for pesticides determination in foodstuff is normally preferred in most laboratories due to the simplicity of determining several pesticides after a single extraction, facilitating the demands of more efficient and rapid monitoring.

Analysis of pesticides in vegetables has for many years been performed by use of gas chromatography (GC) especially coupled with nitrogen phosphorus detector (NPD), electron capture detector (ECD) (Fernández, Picó, & Manes, 2002; Tadeo, Sánchez-Brunete, Albero, & González, 2004) and mass spectrometry (MS) (Beyer & Biziuk, 2008; Ferrer, Garcıa-Reyes, Mezcua, Thurman, & Fernandez-Alba, 2005; Kaihara, Yoshii, Tsumura, Ishimitsu, & Tonogai, 2002; Sánchez-Brunete, Albero, Martín, & Tadeo, 2005; Stajnbaher & Zupancic-Kralj, 2003) or MS/MS (Granby, Andersen, & Christensen, 2004; Hiemstra & de Kok, 2007; Ortelli, Edder, & Corvi, 2004; Sagratini, Manes, Giardina, Damiani, & Pico, 2007; Sannino, Bolzoni, & Bandini, 2004; Soler, Manes, & Picó, 2004). However, high performance liquid chromatography (HPLC) coupled to other less powerful detectors, that are, however, easier to acquire and use (Kaihara et al., 2000; Martínez-Galera, Lopez-López, Gil-García, & Martínez-Vidal, 2001; Paíga, Morais, Correia, Delerue-Matos, & Alves, 2009) is useful and preferable for polar, low volatile and/or thermolabile compounds that are not directly determinable by GC.

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doi:10.1016/j.foodchem.2011.07.137

Analysis of trace levels of pesticides in foodstuff frequently requires extensive extraction and purification procedures to remove high molecular weight interfering compounds such as lipids (Ridway, Lalljie, & Smith, 2007). The extraction process often involves sample preparation, such as, chopping and maceration followed by solvent extraction. Nowadays, extraction methods include solid-phase extraction (Stajnbaher & Zupancic-Kralj, 2003), supercritical fluid extraction (Kaihara et al., 2000), solid-phase microextraction (SPME) (Aulakh, Malik, Kaur, & Schmitt-Kopplin, 2005; Hu et al., 2008), stir bar sorptive extraction (Sandra, Tienpont, & David, 2003) and more recently the 'quick, easy, cheap, effective, rugged, and safe' (QuEChеRS) method (Cunha, Lehotay, Mastovska, Fernandes, & Oliveira, 2007) and matrix solid-phase dispersion (Covaci et al., 2010).

Solid-phase microextraction was introduced and developed by Pawliszyn (Arthur & Pawliszyn, 1990; Pawliszyn, 1997). The technique is based on the partition of the analyte between the sample matrix and a stationary phase that is a fibre coated with an extracting liquid (polymer) or solid (sorbent) phase. Equilibrium is reached between the concentration of the analyte in sample and the amount of analyte sorbed on the fiber, depending on the distribution coefficient. Owing to its convenience, solvent-free operation and low cost, it has gained wide applicability as an analytical technique. To date, SPME coupled with GC has been widely investigated for the analysis of volatile organic compounds including some pesticides (Rodrigues, Reyes, Rehder, & Rath, 2005; Sanchez-Palomo, Diaz-Maroto, & Perez-Coello, 2005). Moreover, SPME coupled with HPLC is receiving increased attention concerning the analysis of non- or semi-volatile, or thermally unstable compounds. Recently, the analysis of pesticides by SPME–HPLC has been reviewed by Aulakh et al. (2005). However, in general the optimised methodologies are applied only to water and biological samples, analyses of pesticides residues in vegetables and fruits are still scarce (Aulakh et al., 2005; Falqui-Cao, Wang, Urruty, Pommier, & Montury, 2001; Wang, Hennion, Urruty, & Montury, 2000). Pesticides use and its residues on lettuce are of particular food safety interest. Lettuce is consumed fresh, so residues that may remain on the harvested product are not removed by processing. Pesticide residues are detected in lettuce and other leafy vegetables more often than in other fresh vegetables. Moreover, lettuce is a major fresh-market vegetable crop (Vandeman, Shank, Chandran, & Vasavada, 1992). The massiveness of the lettuce in European fresh markets is produced in greenhouses where the conditions are favourable to plant growth. However greenhouse mild temperatures and high humidity also promotes fungal and insect development. Key pests in lettuce are the diseases downy mildew and *Botrytis* grey mould and the insects' aphids. Growers prevent these pests using authorised pesticides. In EU the most used pesticides in lettuce are azoxystrobin, acetamiprid, cyprodinil, fenhexamid, fludioxonil, folpet, iprodione, metalaxyl, pirimicarb, and tolyfluanid so they were used in this conduct experiment. To our knowledge, no study has been presented describing the use of SPME and HPLC-DAD for the simultaneous analysis of these pesticides (both fungicides and insecticides) in vegetables.

The development of a SPME–HPLC method requires selection of many variables (fibre type, extraction time, desorption solvent, soaking time, desorption time, extraction temperature, pH of sample solution, and ionic strength) in order to establish optimum conditions. The conventional approach for the optimisation of a multivariable system is usually one-variable-at-a-time. This can be very time consuming and, where interactions exist between the variables, it is unlikely to find the true optimum. Response surface methodology (RSM) is a very useful tool for this purpose as it provides statistical models, which help in understanding the interactions among the parameters that should be optimised (Leardi, 2009). RSM is a collection of mathematical and statistical techniques for modelling and analysis of problems in which a response of interest is influenced by several variables (Ferreira et al., 2007). The main objective of RSM is to determine the optimum operational conditions for the system or to determine a region that satisfies the operating specifications (Ferreira et al., 2007). RSM can be a useful tool for optimisation in analytical chemistry (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008), namely applied to the optimisation of pesticides by SPME (Ferré, Borrull, & Marcé, 1999).

The purpose of this study was the development and validation of a SPME/HPLC-DAD method for multiple pesticide screening in lettuce (*Lactuca sativa*). RSM based on central composite design was used to optimise some variables in SPME. The method was applied to the monitorization of pesticides in greenhouse-grown lettuce samples to study the dissipation behaviour of some of these pesticides during the days to harvest, aiming to evaluate the use of good agricultural practices.

2. Experimental

2.1. Chemical and reagents

All analytical-grade chemicals (purity >98%) including acetamiprid, azoxystrobin, cyprodinil, fenehexamid, fludioxonil, folpet, iprodione, metalaxyl, pirimicarb, tolyfluanid, and phosmet (used as internal standard) were purchased from Sigma–Aldrich (Steinheim, Germany). All the solvents used were HPLC-grade from Merck (Darmstadt, Germany). Ultra-pure water (0.054 µS/cm) was obtained by using a Milli-Q system from Millipore (Milford, MA, USA).

Pesticides standards and calibration solutions were prepared as recommended by SANCO/10684/2009. Stock standards of each pesticide were prepared in acetonitrile and kept from light in a freezer. Working solutions of each pesticide were mixtures of appropriate concentrations prepared weekly and kept at low temperature in the dark.

Different buffers for pH (ranging between 2.64 and 9.36) were prepared to study the effect of pH on SPME pesticide extraction (Perrin & Dempsey, 1974). Buffer with pH 8 selected for analysis of real samples contained 50 mL 0.1 M KH_2PO_4 and 46.1 mL NaOH diluted to 100 mL (Perrin & Dempsey, 1974).

2.2. Sampling strategy

Lettuces (*L. sativa* L. var. *capitata*) were planted in a 300 m^2 plastic greenhouse situated in Póvoa de Varzim, in December 2008. Planting density was 12 plants/m^2 (30 cm × 25 cm). The culture was irrigated whenever necessary with a total of 150 L/m^2. Pesticides were sprayed when decided by grower according to the results of risk assessment for the main pests and diseases. Lettuce plants (1 kg or at least 10 units, depending on the growth phase) were collected before the addition of pesticides (pesticide free samples) and at 0, 1, 3, 6, 7, 9 and 14 days after pesticide addition, respectively t_0, t_1, t_3, t_6, t_7, t_9, t_{14} to study the dissipation behaviour of some of these pesticides after pre-harvest interval in accordance with Directive 2002/63/EC and SANCO/10684/2009. Lettuce samples were immediately transported to the laboratory, freeze for 2 h and mashed with a blender.

2.3. HPLC-DAD conditions

The chromatographic analysis was carried out in an analytical HPLC unit (Jasco, Tokyo, Japan) equipped with Jasco PU-1580 HPLC pumps, a Column Heater (Model 7981; Jones Chromatography, Hengoed, UK), an MD-910 Plus multiwavelength detector. The