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Rapid method based on immunoassay for determination of paraquat residues in wheat, barley and potato

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This work is dedicated to the memory of Dr. Francisco Sanchez-Baeza.

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ABSTRACT

The detection of bipyridine herbicides residues in food samples is hampered due to their particular physico-chemical features, which requires the application of specific extraction and analytical procedures, which disqualifies them from being incorporated into the multi-residue methods (MRMs). There is a need for alternative robust and efficient analytical screening methods, and in this respect, we present here a fast and reliable immunochemical analytical procedure for the detection of paraquat (PQ) residues in food samples, particularly potato, barley and wheat. The procedure involves the extraction with 1 N HCl:MeOH at 80 °C, followed by centrifugation and filtration, and the extracts can be directly measured by a microplate-based ELISA without any other sample treatment or clean-up, except from buffering the solution and adjusting the pH. Selective polyclonal antibodies, were raised against *N*-(4-carboxypent-1-yl)-*N*′-methyl bipyridilium acid (hapten PQ1), and used to establish a high sensitive immunochemical analytical assay, able to measure simultaneously many samples. Under these conditions the accuracy is very good, with almost quantitative recoveries. The non-specific interferences caused by the matrix are negligible for the case of potato and wheat, while for barley it is necessary to further dilute the extract or using a negative certified extract to build the standard calibration curve. The method of extraction consisted in acidic extraction and after a dilution is able to be measured. The analysis method results simply, achieving good detectabilities. The limits of detection (LODs) achieved were between 0.037 ± 0.01 μg kg^{-1} in wheat, 0.71 ± 0.3 μg kg^{-1} in barley and 0.56 ± 0.10 μg kg^{-1} in potatoes, values that are far below the Maximum Residue Level (20 μg kg^{-1}) established by the EU policies for paraquat residues in these foodstuff products. The results demonstrate the high potential of these methods as screening tools for food safety and inspection controls.

1. Introduction

Pesticides are routinely administered throughout the world in order to maximize crop yields during the growth stage and protect agricultural products. However, pesticides may also enter the food production chain through accidental (e.g. contaminated storage containers) or process water contamination and by environment fate. Pesticides are chemicals use to kill, repel, attract, regulate or stop the growth of pests. Among them, there are herbicides, such as the bipyridine herbicides and particularly paraquat (PQ, 1,1′-dimethyl-4,4′-dipyridylium). PQ is a quaternary ammonium compound used as herbicide around the world. Paraquat is a broad-spectrum, non-selective herbicide used for weed control in various crops, as defoliant (cotton, hops) and for destruction of potato haulms (Locke & Wilks, 2001). When absorbed by green shoots, paraquat diverts the energy from sunlight to destroy them (Fernández, Ibáñez, Picó, & Mañes, 1998; Fischer, Rüfenacht, Dannenhauer, Wiesendanger, & Eggen, 2010).

Paraquat is considered one of the most toxic (Erickson, Brown, Wigder, & Gillespie, 1997; Philbey & Morton, 2001; Taylor, Salm, & Pillans, 2001) herbicides in the world. Human epidemiological (Cha et al., 2012; Chester & Woollen, 1982; Hsu et al., 2012; Lee, Bordelon, Bronstein, & Ritz, 2012; Yang et al., 2012) and animals studies with paraquat indicate that paraquat might be an environmental factor contributing to neurodegenerative disorders such as Parkinson's disease (Berry, La Vecchia, & Nicotera, 2010; Brent &

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Schaeffer, 2011; Desplats et al., 2012; Dinis-Oliveira et al., 2006; Fahim, Nemmar, Safa, Adem, & Hasan, 2012; Lee et al., 2012; Yadav, Gupta, Srivastava, Srivastava, & Singh, 2012).

Since July 2007 the use of this pesticide in the EU has been banned, however this does not affect farmers outside the EU from continuing to use PQ and export their products to the EU. Therefore, PQ is included in the EU database between the compounds that should be monitored in food samples and maximum residue limits have been established for different commodities (Commission Regulation, 2008). Thus, for the case of barley, wheat or potato MRLs are 20 μg kg^{-1}. (Commission Regulation, 2011). Following this requirement and in order to protect public health, official laboratories should be able to efficiently process a high number of samples. As a consequence, the development of rapid, cost-effective, sensitive, with high sample throughput and on-site analytical strategies, are required.

The implementation of paraquat within multi-residue methods (MRMs) is difficult due to its physico-chemical properties such as permanent ionic character, high hydrophilicity and a tendency to interact with surfaces, having low recoveries (Guijarro, Yáñez-Sedeño, & Diéz, 1987; Peeters et al., 2001; Startin, Hird, Sykes, Taylor, & Hill, 1999; Winnik et al., 2009). Regarding analytical procedures, high-performance-liquid-chromatography (HPLC) coupled to mass spectrometry (MS) is the most commonly used method (Robb & Eitzer, 2011; Wang, Wang, & Xing, 2011; Whitehead et al., 2010; Wunnapuk et al., 2011). UV detection is also used for certain biological studies (Merritt, Douglas, Rzezniczak, & Watterson, 2011; Zou et al., 2011) although it presents certain limitations due to the particular physico-chemical properties of paraquat. A surface-enhanced Raman scattering (SERS)-based microdroplet sensor has been developed with excellent detection limits, although its application to the analysis of residues in complex samples has not been demonstrated (Gao et al., 2010). Moreover, a distinct cell-based biosensors systems, based on the toxicity of this pesticide, has been reported (Kim, Youn, Ahn, & Gu, 2005; Lee & Gu, 2005; Lee, Mitchell, Kim, Cullen, & Gu, 2005; Podola & Melkonian, 2005; Strachan, Capel, Maciel, Porter, & Paton, 2002), but the detectability achieved is not suitable for screening residues in food products. All these techniques either do not reach the necessary detectability or are time-consuming, since laborious sample extraction, concentration or clean-up procedures are required to perform accurate and reliable measurements. Moreover, it often involves sophisticated or complex equipment which increase the cost of routine analytical screening programs. As useful complementary methods, immunochemical analytical techniques, including immunoassays (Bacigalupo, Meroni, Mirasoli, Parisi, & Longhi, 2004; Bowles, Eyles, Hampson, & Pond, 1992; Coxon, Rae, Gallacher, & Landon, 1988; Selisker, Herzog, Erber, Fleeker, & Itak, 1995; Spinks, Wang, Mills, & Morgan, 1999; Van Emon, Seiber, & Hammock, 1987) and immunosensors (Mallat, Barzen, Abuknesha, Gauglitz, & Barceló, 2001; Mastichiadis et al., 2002) have been developed and used for the quantification of paraquat in a variety of environmental and biological matrices. The efficiency and potential capabilities of the immunochemical techniques, in their wide variety of configurations, as reliable screening tools has been demonstrated during the last two decades (Adrian et al., 2009; Conzuelo et al., 2012; Farré, Ramón, Galve, Marco, & Barceló, 2006; Fernández, Pinacho, Sanchez-Baeza, & Pilar Marco, 2011; Fernández, Sánchez-Baeza, & Marco, 2012; Nichkova & Marco, 2006; Salvador, Sánchez-Baeza, & Marco, 2010). In order to establish reliable immunochemical analytical methods, the development of compatible extraction methods and knowledge of the potential interferences caused by the matrix is crucial. Thus, in very few occasions paraquat residues have been detected by immunochemical analytical techniques food matrices. Consequently, the purpose of our work is to establish a high-throughput immunochemical screening methods for the analysis of paraquat residues in food samples, in which the presence of this pesticide is likely possible, in compliance with the EU requirements in respect to the MRLs for paraquat. With this purpose, we report here the production of antibodies for this pesticide, the development of an immunoanalytical procedure, and the establishment of a whole immunochemical analytical protocol for the analysis of pesticides residues in cereals and potato samples.

2. Materials and methods

2.1. Synthesis

The synthesis of Paraquat (PQ) and Monoquat (MQ) was performed reacting 4-4′-bipyridine with iodomethane according to the described procedures by Abuknesha and Luk (2005). PQ1 hapten was obtained by reacting MQ with methyl iodovalerate as previously described (Abuknesha & Luk, 2005; Van Emon, Hammock, & Seiber, 1986). The product obtained was hydrolyzed in acid condition to obtain the final product which was purified and characterized by ^{1}H, ^{13}C NMR and exact MS (see Supplementary material).

2.2. Immunochemistry: general methods and instruments

The matrix-assisted laser desorption/ionisation mass spectrometer (MALDI-MS) used for analyzing the protein conjugates was performed on a time-of-flight (TOF) mass spectrometer (Bruker Autoflex III, smart-wing; Bruker, Bremen, Germany). The pH and conductivity of all buffers and solutions were measured with a pH-meter (pH 540 GLP) and a conductimeter (LF 340), respectively (both from WTW, Weilheim, Germany). Polystyrene microtiter plates were purchased from Nunc (Maxisorp, Roskilde, Denmark). The vacutainer blood collection set was acquired from Becton Dickinson (Meylon Cédex, France). Washing steps were performed on a SLY96 PW microplate washer (SLT Labinstruments GmbH, Salzburg, Austria). UV spectra and absorbances were read on a Molecular Devices (Sunnyvale, CA) SpectramaxPlus spectrometer with SoftmaxPro v4.7 software. Competitive curves were analyzed with a four-parameter equation using GraphPad Software, Inc. (San Diego, CA) and GraphPad Prism 4 software. The filters were Millex-GN 0.20 μm filters (Millipore, Carrigtwohill, County Cork, Ireland). Unless otherwise indicated, the data presented correspond to the average of at least two well replicates.

2.3. Chemicals and immunochemicals

Chemicals were acquired from Aldrich Chemical Co. (Milwaukee, WI). Horseshoe crab hemocyanin (HCH), bovine serum albumin (BSA), ovalbumin (OVA), conalbumin (CONA), and other biochemical reagents were from Sigma Chemical Co. (St. Louis, Missouri). Standards for cross-reactivity studies were supplied by different sources. Paraquat, monoquat and diquat, used as standard, were prepared in our laboratory 2,2′-bipyridine and 4,4′-bipyridine were obtained from Sigma Chemical Co. (St. Louis, MO). Atrazine, Irgarol 1051 and 2,4,6-trichloropyridine were kindly supplied by Prof. Damià Barceló from IDAEA-CSIC (Barcelona, Spain). Sulfapyridine, was supplied by Riedel-de Haën (Buchs, Switzerland). Stock solutions of each analyte (10 mmol L^{-1}) were prepared in dimethyl sulphoxide (DMSO) and stored at 4 °C. The preparation of the protein conjugates and the antisera is described below.

2.4. Buffers and solutions

The phosphate-buffered saline solution (10 mM PBS; pH 7.5) contained 2 mM KH_2PO_4 and 8 mM Na_2HPO_4 in a 0.8% saline