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Routine application using single quadrupole liquid chromatography–mass spectrometry to pesticides analysis in citrus fruits

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Abstract

A rapid and sensitive liquid chromatography–electrospray ionization–mass spectrometry method has been developed for the routine analysis of buprofezin, bupirimate, hexaflumuron, tebufenpyrad, fluvalinate and pyriproxyfen in citrus fruits. Extracts were obtained by matrix solid-phase dispersion (MSPD) using C_{18} as dispersant and dichloromethane-methanol (80:20, v/v) as eluent. Matrix effects were tested for all matrices by addition of standard to sample blank extracts (samples containing no detectable residues). Mean recoveries obtained at fortification levels between 0.01 and 5 mg kg^{-1} were 57–97% with relative standard deviations (RSDs) from 5 to 19%. The limits of quantification (LOQ) were in the range of 0.01–0.2 mg kg^{-1} and lower than maximum residue limits (MRLs) established by the Spanish legislation. The MSPD was compared with conventional ethyl acetate extraction, showing equivalent recoveries and precision. Although the sample is more concentrated (5-fold) by solid–liquid extraction (SLE) with ethyl acetate than by MSPD, LOQs obtained by both techniques, were almost equal, because MSPD reduces matrix effects, baseline noise, and interfering peaks from the matrix. The proposed method has been applied to the determination of selected pesticides in real samples. Liquid chromatography–tandem mass spectrometry (LC–MS–MS) with quadrupole ion trap (QIT) and triple quadrupole (TQ) have been used as confirmatory tool for positive samples according to a recent No. SANCO/10476/2003 European Union Guideline.

Keywords: Food analysis; Citrus fruits; Pesticide residues

1. Introduction

Use of agrochemicals at various stages of cultivation and during post-harvest storage, plays an important role in food protection and quality preservation. However, widespread-used pesticides become a very important group of chemicals to be controlled because of their high toxicity to the human health and frequent presence of their residues in fruits and vegetables [1,2]. One of the most important aspects for minimizing the potential hazards to humans is the monitoring of pesticide residues in food. Maximum residue limits (MRLs) in fruits and vegetables have been set by the governmental agencies of each country [3,4] and the European Union (EU) [5] to guarantee consumer safety and to minimize their consumers' intake.

Analytical methodologies employed, owing to the strict regulation of MRLs, must be capable of residues measuring at trace levels [6,7] and of providing unambiguous evidence to confirm both, the identity and the quantity of any detected pesticide [8]. These routine methods should be simple, fast, and robust to minimize time spent per sample [9]. In the last decades, the on-line coupling of efficient liquid chromatography separation with mass spectrometry detector (LC–MS) has been used for the analysis of pesticide residues [10,11] and is rapidly becoming an accepted technique for regulatory monitoring purposes [12,13].

Advantages of the LC–MS are the reduction of sample preparation steps that provides a higher sample throughput and the high sensitivity and selectivity that enable the

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analysis of target analytes at low concentrations. Main disadvantage of this technique is that using single quadrupole, an alternative technique is required to meet the European Union identification criteria established in the document No. SANCO/10476/2003 [14].

LC in combination with tandem mass spectrometry (LC–MS–MS) has provided the most powerful confirmatory tool for the pesticide residue analysis in food [15] because it discriminates more efficiently than LC–MS between the analyte and the matrix signal and it is especially relevant when the ultra-trace levels identification is needed [16]. Several multiresidue screening methods have been developed for routine application [17,18]. However, LC–MS–MS has the disadvantages for a routine analysis in the laboratories of being very expensive, requiring high-pure gas for collision-activated dissociation (CAD), having expensive replacements and being very delicate in its adjusting.

The common established extraction techniques are based on complex solvent extraction methods that for solid samples are also named solid–liquid extraction (SLE). These procedures have some drawbacks such as: they are time consuming, require high amount of sample and solvents, and lack sufficient specificity to avoid false positives [19]. That is the reason why they are replaced with faster, less expensive and easy handled protocols. Matrix solid-phase dispersion (MSPD) carries out simultaneously sample homogenization, extraction and clean-up [20] using a relative small sample size, low solvent volume and minimum amount of sample. In the last years, it has been increasingly applied for isolating pesticides from fruits and vegetables [19–21].

The aim of this work was to develop a rapid, specific and sensitive analytical method for the routine analysis of six widely used pesticides in citrus fruits at concentration levels lower than their respective MRLs. These pesticides have been scarcely studied previously. It involves a rapid and low time-consuming MSPD extraction that accomplished high sample throughput and routine determination of the sample using LC–MS with single quadrupole monitoring the main ion obtained for each analyte ($[M+H]^+$ or $[M+Na]^+$). The confirmation of positive samples was performed by LC–MS–MS using either triple quadrupole (TQ) or quadrupole ion trap (QIT) to meet the European Union requirements.

2. Experimental

2.1. Materials and standards

Pesticides (buprofezin, bupirimate, hexaflumuron, pyriproxifen, tebufenpyrad and fluvalinate) were supplied by Riedel-de Haën (Seelze, Germany). Individual stock solutions were prepared dissolving 10 mg of each compound in 10 ml of methanol and stored in stained glass-stopper bottles at 4 °C. Standard working mixtures for each pesticide at various concentrations were daily prepared by appropriate dilution of aliquots of the stock solutions in methanol or in matrix extract.

HPLC-grade methanol, ethyl acetate and dichloromethane (organic trace analysis) were purchased from Merck (Darmstadt, Germany). Deionized water (<18 mol cm^{-1} resistivity) was obtained from the Milli-Q SP Reagent Water system (Millipore, Bedford, MA, USA). All the solvents and solutions were filtered through a 0.45 μm cellulose filter from Scharlau (Barcelona, Spain) before use.

The solid phase for MSPD was C_{18} bonded silica (40–60 μm) from Analisis Vinicos (Tomelloso, Spain).

2.2. Sample preparation

Oranges, tangerines, grapefruits and lemons used as blank samples (samples with no detectable residues) and as spiked ones were from organic farming without use of pesticides and obtained from a local market. The developed procedure was also applied to the analysis of 80 samples that were taken, at random, out of those conventionally farmed. The samples were taken in accordance with the guidelines of the EU [5]; which means that, as far as possible, the sample was taken at various places distributed throughout the lot (size ca. 50 kg). The samples, weighting at least 1 kg, consisted of 10 individual fruits, were immediately stored in polyethylene bags for transporting to the laboratory. Samples were stored at 4 °C until the moment of extraction and analyses were carried out for the next 24 h to avoid problems of stability during the storage.

They were analyzed unwashed and unpeeled because Spanish legislation establishes the MRLs in mg kg^{-1} of whole sample. A representative portion of sample (200 g whole fruit) was chopped into small pieces and homogenized in a Bapitaurus food chopper (Taurus, Berlin, Germany). Two subsamples (30–40 g) of these representative portions were stored at −20 °C because it was necessary to repeat the analysis. No degradation of the pesticides, when they are present, was detected under these conditions.

2.2.1. Matrix solid-phase dispersion procedure

Portions of 0.5 g of chopped sample were weighed, placed into a glass mortar (50 ml) and gently blended with 0.5 g of C_{18} bonded silica for 5 min using a pestle, to obtain homogeneous mixture.

The homogeneous mixture was introduced into a 100 mm × 9 mm I.D. glass column, and eluted dropwise with 10 ml of a dichloromethane-methanol (80:20, v/v) mixture by applying a slight vacuum. The eluated was collected in a graduated conical tube (15 ml capacity) and concentrated, under stream of nitrogen, to 0.5 ml. An aliquot of 5 μl of the final extract was injected into the LC apparatus.

2.2.2. Solid–liquid extraction procedure employing ethyl acetate

Fifty grams of chopped sample placed in a 250 ml glass beaker were mixed thoroughly with 100 ml of ethyl acetate