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Determination of 23 pesticide residues in leafy vegetables using gas chromatography–ion trap mass spectrometry and analyte protectants

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ABSTRACT

A gas chromatographic method was developed for simultaneously determining residues of 12 insecticides (acrinathrin, bifenthrin, carbofuran, cyfluthrin, λ-cyhalothrin, cypermethrin, chlorfenvinphos, deltamethrin, esfenvalerate, fenamiphos, methiocarb and τ-fluvalinate) and 11 fungicides (cyprodinil, fludioxonil, iprodione, metalaxyl, penconazole, pyrimethanil, procymidone tebuconazole, triadimefon, triadimenol and vinclozolin) in leafy vegetables. Samples were extracted with acetonitrile and cleaned-up with graphitized carbon black/primary secondary amine (GCB/PSA) solid-phase extraction (SPE) cartridges using acetonitrile:toluene (3:1, v/v) as eluent. The eluate was finally evaporated and redissolved with 0.5 mL of acetone containing the internal standards (pentachlorobenzene and fenpropathrin) and three analyte protectants (3-ethoxy-1,2-propanediol, D-sorbitol and L-gulonic acid γ-lactone). The addition of analyte protectants allows to avoid the matrix-induced response enhancement effect on quantitation process with absolute recoveries *ca.* 100%. Precision (expressed as relative standard deviation) was lower than 10% for all pesticides and finally, limits of detection were also 10–20 times lower than maxima residue levels (MRLs) established by European Regulation. The proposed method was applied to determine pesticide residues in commercial leafy vegetables (lettuce, Swiss chard and spinach) purchased from markets in Orense (NW Spain). Pesticide residues were detected in 84% of the total samples (63 from 75 samples) and pesticide concentrations were higher than MRL in 18 samples.

1. Introduction

The application of pesticides to agriculture has greatly improved the food production worldwide. European Regulation 396/2005 and amendments are establishing shortly the maximum quantities of pesticide residues permitted in products of animal or vegetable origin that are intended for human or animal consumption [1]. The objective is to ensure that pesticide residues in foodstuffs do not constitute an unacceptable risk for consumer and animal health. Currently, maximum residue limits (MRLs) in force for the following foodstuffs are established in Directives 76/895/EEC (fruit and vegetables), 86/362/EEC (cereals), 86/363/EEC (foodstuffs of animal origin), 90/642/EEC (products of vegetable origin and honey) and amendments [2–5].

Pesticide residue analysis in agricultural commodities compared to other organic trace analysis has some peculiarities: (i) a wide range of analytes, with different polarities, solubilities and pK_a values, and at different concentrations levels, may be determined in the same sample; (ii) there is a wide range of commodities with different matrix effects in the determination of analytes due to different water and fat content and biochemical composition; (iii) Certified Reference Materials (CRMs) are not available.

Pesticide residue analysis is routinely carried out by means of multi-residue methods based on homogenization of the sample with an appropriate solvent, separation of the liquid portion of the sample from insoluble material, purification and clean-up by solid-phase extraction (SPE) followed by a final chromatographic determination step. Organic solvents commonly used to extract pesticide residues in fresh fruits and vegetables are acetonitrile, acetone and ethyl acetate [6–8]. An extensive clean-up of organic extracts is necessary to reduce adverse effects related to the quantification of residues such as the masking of residue peaks by coeluted matrix component, the occurrence of false positives and/or the inaccurate quantitation. Clean-up procedures in fresh fruits and vegetables use a combination of two or more commercially available SPE columns in the normal-phase mode. Weak anion-exchange sorbents such as primary secondary amine (PSA), aminopropyl (NH_2) or diethylaminopropyl (DEA) modified silica are often used for clean-up of food samples together with strong anion-exchange sorbents (SAX, QMA) [9]; other SPE

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clean-up approaches include the combination of graphitized carbon black (GCB) and PSA columns [10,11], the combination of C_{18}, GCB and NH_2 [12] and finally the combination of GCB, PSA and SAX columns [13]. Alumina, fluorisil and silica SPE columns are not commonly used in multi-residue methods because they strongly adsorb polar organophosphorus pesticides [14]. Different approaches on quantitation of pesticide residues in fresh fruits and vegetables can be considered in order to minimize the matrix effect: (i) use of the standard addition method; (ii) use of standards in residue-free matrix spiked with standards (matrix-matched standards); (iii) use of deuterated internal and/or surrogate standards and/or (iv) compensation of the calculated results by a matrix factor.

The main aim of this work is the development of an accurate interference-free method for the joint determination of multiple pesticides belonging to 8 different chemical groups (pyrethroids, carbamates, aniline-pyrimidines, organophosphates, phenylpyrroles, dicarboximides, phenylamides and triazoles) in Spanish commercial vegetables. For this purpose, a programmable temperature vaporization injector (PTV)–gas chromatography coupled with an ion trap mass spectrometry detection system (GC–ITMS) was used; ITMS was selected as detector because of its ability to perform simultaneous quantitative and qualitative analysis of trace level compounds. Different types of SPE cartridges were evaluated in order to determine the relative degree of sample clean-up.

2. Experimental

2.1. Chemicals and reagents

Pesticides tested were: acrinathrin, bifenthrin, carbofuran, cyfluthrin, λ-cyhalothrin, cypermethrin, cyprodinil, chlorfenvinphos, deltamethrin, esfenvalerate, fenamiphos, fludioxonil, iprodione, metalaxyl, methiocarb, penconazole, pyrimethanil, procymidone, tau-fluvalinate, tebuconazole, triadimefon, triadimenol and vinclozolin. Methiocarb standard, of certified purity of 99%, was obtained from Supelco (Bellefonte, PA, USA); all the rest were pestanal grade standards, of certified purity >98%, obtained from Riedel-de-Haën (Seelze, Germany). Fenpropathrin (Pestanal grade, purity of 98.3% from Riedel-de-Haën) and pentachlorobenzene (purity of 99.5% from Dr. Ehrenstorfer, Augsburg, Germany) were used as internal standards to correct for variability in gas chromatographic injection and mass spectrometric detection response. The 3-ethoxy-1,2-propanediol (98%), D-sorbitol (>99%) and L-gulonic acid γ-lactone of (>98%), used as analyte protectants, were obtained from Aldrich (Steinheim, Germany).

Solvents (residue analysis grade) used were acetone, acetonitrile, toluene (Panreac, Barcelona, Spain) and methanol (Scharlau, Barcelona, Spain). Other reagents such as sodium chloride and anhydrous magnesium sulphate for residue analysis were also purchased from Panreac.

2.2. Stock standard solutions

Individual stock standard solutions (ca. 1000 mg/L) of pesticides and internal standards were prepared by dissolving 25 mg of each compound in 25 mL of methanol; acetone substituted methanol to dissolve acrinathrin, cyfluthrin, fenamiphos, iprodione and tau-fluvalinate. Bifenthrin was supplied as a solution (100 mg/L, in acetonitrile); following dilutions of this insecticide was performed in acetone. All standard solutions were stored in glass-stoppered flasks at 4 °C. Mixed compound calibration solutions were prepared in acetone and they were used as spiking solutions.

Individual stock standard solutions of analyte protectants – 3-ethoxy-1,2-propanediol, D-sorbitol and L-gulonic acid γ-lactone – were prepared in acetonitrile (50 g/L), acetonitile:water (85:15, v/v, 5 g/L) and acetonitrile:water (80:20, v/v, 5 g/L), respectively.

2.3. Materials for solid-phase extraction and small apparatuses

The sorbent materials used for solid-phase extraction were as follows: strata NH_2– (500 mg, 6 mL size) and X–AW (500 mg, 6 mL size) from Phenomenex (Torrance, CA, USA); Supelclean PSA (300 mg, 6 mL size), Supelclean Envi-Carb (500 mg, 6 mL size), Supelclean SAX/PSA (500 mg/500 mg, 6 mL size) and Supelclean Envi-Carb II/PSA (500 mg/500 mg, 6 mL size) from Supelco (Bellefonte, PA, USA).

For solid–liquid extraction, samples were placed in 125 mL glass containers from Afora (Barcelona, Spain). Organic extracts were placed in round-bottomed flasks from Schott Duran (Mainz, Germany) prior to evaporation on a Heidolph WB 2000 vacuum rotary evaporator (Schwabach, Germany). The final extracts were homogenized by vortex shaking on a Heidolph Reax Top apparatus and placed via 350 μL glass inserts into 2 mL vials from Supelco prior to chromatographic analysis.

2.4. Pesticide residue determination

Pesticide residue determination in leafy vegetables, once optimized, involved a solid–liquid extraction (SLE)/SPE/PTV–GC–ITMS with the following instrumental conditions:

2.4.1. Extraction process

The extraction and clean-up procedures have been described elsewhere [10]. The chopped vegetable samples (10 g) were placed in a 125 mL glass container and extracted with 30 mL of acetonitrile. The glass container was vigorously homogenized in an ultrasound bath for 10 min. Sodium chloride (3 g) and anhydrous magnesium sulphate (12 g) were added followed by vigorous shaking for 5 min and phase partitioning for 10 min; magnesium sulphate rather than sodium sulphate was used because it has been shown that the last salt is relatively ineffective in removing water from acetone:water or acetonitrile:water mixtures [15]. An aliquot of 15 mL of the organic layer was transferred to a 100 mL round-bottomed flask and evaporated to 1–2 mL at 40 °C on the rotary evaporator (220 mbar).

2.4.2. Clean-up process

For the sample clean-up, multi-layer Supelclean Envi Carb-II/PSA SPE cartridge was conditioned with 5 mL of acetonitrile:toluene (3:1, v/v). Acetonitrile extract was loaded and the retained pesticides were eluted slowly, in a 50 mL round-bottomed flask, with a volume of 20 mL of acetonitrile:toluene (3:1, v/v). The eluate was evaporated to dryness (40 °C, 75 mbar) and the solvent was substituted with 0.5 mL of acetone containing 0.5 mg/L of each internal standard (pentachlorobenzene and fenpropathrin) and the three analyte protectants (10 g/L of 3-ethoxy-1,2-propanediol, 1 g/L of each D-sorbitol and L-gulonic acid γ-lactone). The final acetone extract was homogenized with vortex agitation.

2.4.3. Chromatographic analysis

Gas chromatographic (GC) analyses were carried out on a Trace GC Thermo Finnigan gas chromatograph (Rodano, Italy) equipped with a PolarisQ ITMS detection system, interfaced to a PC computer running the software XCalibur 1.4, from Thermo Electron Corporation (Italy). Chromatographic separations were done by using a SPB-5 fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm