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Planar solid phase extraction clean-up and microliter-flow injection analysis–time-of-flight mass spectrometry for multi-residue screening of pesticides in food

Claudia Oellig*, Wolfgang Schwack

Institute of Food Chemistry, University of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Germany

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ABSTRACT

For multi-residue analysis of pesticides in food, a sufficient clean-up is essential for avoiding matrix effects in liquid and gas chromatography (LC and GC) analysis coupled to mass spectrometry (MS). In the last two years, high-throughput planar solid phase extraction (HTpSPE) was established as a new clean-up concept for pesticide residue analysis in fruits and vegetables (C. Oellig, W. Schwack, 2011) and tea (C. Oellig, W. Schwack, 2012). HTpSPE results in matrix-free extracts almost free of interferences and matrix effects. In this study, a time-of-flight mass spectrometer (TOFMS) was applied to directly analyze HTpSPE extracts for pesticide residues. This HTpSPE–microliter-flow injection analysis (μL-FIA)–TOFMS approach detects all pesticides at once in a single mass spectrum, without a liquid chromatographic separation step. Complete sample information was obtained after the injection of the cleaned extract within a single peak. Recovery studies for seven representative pesticides in four different matrices (apples, red grapes, cucumbers, tomatoes) provided mean recoveries of 86–116% with relative standard deviations of 1.3–10% ($n = 5$) using the mass signal intensities under the entire sample peak. Comparing the mass spectra of sample peaks from spiked extracts and solvent standards indicated the efficiency of HTpSPE clean-up. A pesticide database search detected all spiked pesticides with a low incidence of false-positives. HTpSPE of one sample required a few minutes, and numerous samples could be cleaned in parallel at minimal cost with low sample and solvent consumption. The μL-FIA–TOFMS screening then needed an additional 6 min per sample. The novel screening approach was successfully applied to QuEChERS extracts of several real samples, and the pesticides identified by HTpSPE–μL-FIA–TOFMS were identical to the pesticides detected by common target LC–MS/MS analyses. The high degree of concordantly identified pesticides by the new developed HTpSPE–μL-FIA–TOFMS approach and target LC–MS/MS demonstrates the applicability as a routine screening method.

1. Introduction

Pesticide residue analysis is generally performed in a series of steps. After the extraction of target analyte(s) from the sample matrix with organic solvents, extract clean-up and concentration (as needed) followed by liquid or gas chromatography (LC or GC) coupled to a mass selective detector provides a high degree of selectivity and sensitivity. Frequently used analyzers for LC–MS and GC–MS systems are single-quadrupole mass selective detectors (MS) [1–7], triple-quadrupole (QqQ) [5–22], ion trap (IT) [6,23–28] or quadrupole linear ion trap (QqLIT) systems [6,7,10,29], with QqQ, IT and QqLIT operated in the selected reaction monitoring mode (SRM). Advantages of the target tandem MS (MS/MS) operating in SRM are high sensitivity and selectivity based on analyte-specific ions and transitions which are strongly target-oriented. This limitation excludes pesticides which might be in the sample, but not in the focus of the method [6,7,9,30], and is the main drawback of the target MS/MS detection mode. Nevertheless, LC–MS/MS and GC–MS(/MS) are the techniques of choice for pesticide residue analysis and are often called the "workhorses" in target analysis [31].

However, more and more research is focused on high-resolution MS (HRMS) like time-of-flight (TOF) and quadrupole time-of-flight (QqTOF) [6,7,10,30–48], as well as the desktop Orbitrap system [6,34,49–53] used as highly selective detectors for LC. They are applied to a database-supported target screening [30–33,37,40,41,43,45,48–50], and also allow screening for unknowns in terms of a non-target-oriented analysis

* Corresponding author. Tel.: +49 711 459 24098; fax: +49 711 459 24096.
E-mail address: claudia.oellig@uni-hohenheim.de (C. Oellig).

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[30–32,39,42,46,48,50,53–56] including "retrospective data analysis" [49] as the full-scan spectra information is saved. While highly attractive, publications are not available showing results of a flow injection analysis (FIA)–HRMS approach which omits chromatographic separation to rapidly screen for residues and contaminants. Instead, MS/MS was used for an "extract and shoot" FIA technique to perform rapid screenings [57–60].

However, these attempts are subject to one of the most serious problems in trace analysis of biological and environmental samples, the so-called "matrix effect", identified as the major source of uncertainty in LC–MS and GC–MS [61–64] which are due to different mechanisms [61–63,65]. Depending on the matrix type, they account for (i) false-negatives, (ii) false-positives, (iii) inexact quantitation [62,63] caused by ion suppression or ion enhancement, or (iv) retention time-shiftings, especially during GC [62]. On the other hand, high amounts of co-extracted matrix compounds can contaminate the analytical instruments. Therefore, the magic bullet for rapid, accurate, precise and robust analyses is an efficient clean-up leading to matrix-free samples to be analyzed by LC–MS or GC–MS.

Various methods exist for sample clean-up depending on the materials, including gel permeation chromatography (GPC) [66–68], cartridge solid phase extraction (SPE) [15,22,68] or dispersive solid phase extraction (dSPE) [13,68] to remove fatty acids, lipids, phenols, chlorophyll and other co-extracted matrix compounds from fruits and vegetables [22,69–72]. However, these methods are only partly successful, sensitive towards loss of pesticides [63,68,73], and are subject to errors, which is why some compromises have to be made.

To compensate for residual matrix effects, matrix-matched calibration standards are generally applied [13,20,21,41,59,61–63,65,74–76], while some single-residue methods use expensive stable isotope-labelled internal standards for calibration to overcome matrix effects [62,63,75,77,78]. Further attempts to reduce these effects by calibration techniques are the standard addition method [6,63,68,79–82], the echo-peak technique [63,75,77,83–85], the post-column infusion method [86–88] and the addition of "analyte protectants" for GC–MS [6,89–95]. Another way to overcome matrix effects is the dilution approach [57,62–64,77,80,96–101], which thanks to the increasing sensitivity of MS systems becomes more and more popular as they are rapid, easy and inexpensive.

High-throughput planar solid phase extraction (HTpSPE) is a recently developed efficient clean-up method for residue analysis. Instruments of high-performance thin-layer chromatography (HPTLC) [102], with their benefits to detect nearly everything on the TLC plate, were combined with low-cost and rapid side-by-side sample analyses under repeatable conditions and high automation [103], replacing SPE and GPC. HTpSPE resulted in matrix-free extracts almost free of interference and matrix effects. This technique combined a fully automated sample application and plate development with the TLC–MS interface as the essential tools of the method. This feasible, easy and rapid clean-up method was successfully used and yielded reliable and highly reproducible results for fruit and vegetable matrices [104] as well as tea samples [105] by LC–MS(/MS).

In an effort to extend the scope of the HTpSPE, a new screening approach was developed for pesticide residue analysis of fruits and vegetables. HTpSPE clean-up was combined with a microliter (μL)-FIA–TOFMS mass analyzer system, omitting the liquid chromatographic separation step, which only was promising due to the matrix-free sample extracts. As nanospray ionization additionally reduces matrix effects [63,106,107] and offers low solvent consumption, a nanospray interface combined with a μL-flow rate was used. After developing optimal configurations for the μL-FIA–TOFMS measurements which included optimizing the liquid flow and injection parameters, nanospray ionization and detector settings, the method was applied to several fruit and vegetable samples. In addition, a database search tool based on Microsoft EXCEL and ACCESS was developed for target and non-target screenings with the obtained full-scan HRMS data.

2. Materials and methods

2.1. Chemicals and materials

Azoxystrobin, fenarimol and mepanipyrim were purchased from Ehrenstorfer (Augsburg, Germany), and chlorpyrifos, pirimicarb and Sudan II from Sigma–Aldrich (Steinheim, Germany). Acetamiprid, penconazole and the internal standard (ISTD) tris(1,3-dichloro-2-propyl)phosphate (TDCPP) were received from High Purity Compounds (Cunnersdorf, Germany). Aspartame was purchased from NutraSweet AG (Zug, Switzerland), brucine (purum, 97%) from Fluka (Buchs, Switzerland), caffeine (USP/BP, 98.5%) from Acros Organics (Geel, Belgium), lidocaine (reagent grade) from Sigma-Aldrich and reserpine (99%) from Alfa Aesar (Karlsruhe, Germany). Sodium chloride (pro analysis) and di-sodium hydrogencitrate 1.5-hydrate (>99%) were obtained from Merck (Darmstadt, Germany), and sodium citrate tribasic dihydrate (>99%) and magnesium sulphate, anhydrous (reagent grade, ≥97%) from Sigma–Aldrich. Bondesil–PSA (primary secondary amine, 40 μm), was purchased from Varian (Palo Alto, USA). Acetone (Rotisolv pestilyse) was obtained from Carl Roth (Karlsruhe, Germany). Acetonitrile and methanol (both LC–MS, Chromasolv), formic acid (for LC–MS, ~98%) and ammonium formate (for mass spectrometry, ≥99.0%) were purchased from Sigma–Aldrich. Ultrapure water (>18 MΩ cm) was supplied by a Synergy System (Millipore, Schwalbach, Germany). TLC aluminium foils silica gel 60 NH_2 F_{254}s, 20 cm × 20 cm, with a layer thickness of 0.15–0.18 mm purchased from Merck were prewashed two times with acetonitrile and dried at room temperature inside a fume-hood for 15 min. The foil was vertically cut at 10 cm, and both 20 cm × 10 cm halves were stored in a desiccator until use.

2.2. Solutions

Standard stock solutions of pesticides at a concentration of 500 μg/mL were prepared in acetonitrile. For the ISTD stock solutions, TDCPP and Sudan II were dissolved in acetonitrile at a concentration of 500 and 100 μg/mL, respectively. Sudan II was used as a visible marker for the target TLC zone (pesticides). The stock solutions were stored at −20 °C.

The spiking solution for recovery experiments was prepared by mixing and diluting stock solutions with acetonitrile, resulting in 5 μg/mL concentrations for each pesticide. For mass spectra data comparison and database searching, respective dilutions of the stock solutions were prepared, resulting in a spiking solution containing 5 μg/mL acetamiprid, azoxystrobin, mepanipyrim and pirimicarb, 10 μg/mL penconazole, and 30 μg/mL fenarimol and chlorpyrifos. The ISTD stock solutions were generally diluted with acetonitrile to a concentration of 150 μg/mL TDCPP and 20 μg/mL Sudan II.

2.3. Samples and extraction

As representative fruit and vegetable matrices, organically produced apples, red grapes, tomatoes and cucumbers were obtained from a local supermarket and checked to be free of the selected pesticides by LC–MS and μL-FIA–TOFMS measurements of dSPE and HTpSPE extracts. Food samples were cut into pieces, ground (GRINDOMIX GM 300 knife mill, Retsch, Haan, Germany) and the citrate buffered QuEChERS method [13] was used as a guideline for sample extraction. In brief, 10 g of ground sample was weighted