Journal of Chromatography A, 1328 (2014) 66–79

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Application of ultra-high pressure liquid chromatography linear ion-trap orbitrap to qualitative and quantitative assessment of pesticide residues

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ARTICLE INFO

Article history:
Received 30 September 2013
Received in revised form
24 December 2013
Accepted 26 December 2013
Available online 2 January 2014

Keywords:
UHPLC
LTQ-Orbitrap-MS
Pesticide quantification
Food samples
Environmental samples
Contaminants identification

ABSTRACT

The analysis of pesticides residues using a last generation high resolution and high mass accuracy hybrid linear ion trap-Orbitrap mass spectrometer (LTQ-Orbitrap-MS) was explored. Pesticides were extracted from fruits, fish, bees and sediments by QuEChERS and from water by solid-phase with Oasis HLB cartridges. Ultra-high pressure liquid chromatography (UHPLC)–LTQ-Orbitrap mass spectrometer acquired full scan MS data for quantification, and data dependent (dd) MS^2 and MS^3 product ion spectra for identification and/or confirmation. The regression coefficients (r^2) for the calibration curves (two order of magnitude up to the lowest calibration level) in the study were ≥0.99. The LODs for 54 validated compounds were ≤2 ng mL^{-1} (analytical standards). The relative standard deviation (RSD), which was used to estimate precision, was always lower than 22%. The recovery of extraction and matrix effects ranged from 58 to 120% and from −92 to 52%, respectively. Mass accuracy was always ≤4 ppm, corresponding to a maximum mass error of 1.6 millimass units (mmu). This procedure was then successfully applied to pesticide residues in a set of the above-mentioned food and environmental samples. In addition to target analytes, this method enables the simultaneous detection/identification of non-target pesticides, pharmaceuticals, drugs of abuse, mycotoxins, and their metabolites.

1. Introduction

The analysis of pesticide residues in a variety of food and environmental matrices contributes to ensure their safety and quality and to guarantee health standards [1]. Multi-residue analysis of these compounds at trace levels has been carried out since the 70s using mostly gas chromatography (GC) with element selective detectors [2–4]. Although these detectors were sensitive enough, they provided poor specificity and were gradually replaced by GC–mass spectrometry (GC–MS), which is still a standard analytical tool thanks to the availability of spectra libraries and appropriate deconvolution software [3–6]. However, currently used pesticides are quite polar, thermally labile or not easily vaporized and, consequently, are better determined using liquid chromatography (LC) also combined with MS [7,8]. The principles of mass detection also vary, with the most common instruments being triple quadrupole, ion trap, hybrid linear ion-trap (QTRAP) and (quadrupole) time of flight [(Qq)TOF] mass spectrometers [9–13].

While discussion of the merits of each type of chromatography, ion source, and mass detector are beyond the scope of this paper, it is evident that many different types of applications can be developed with LC–MS [10,11,14]. An increasingly utilized type of LC–high resolution (HR)MS applications is screening and profiling [15–17], where the extraction, LC methods, and MS instrument setup are set to provide a broad coverage of compounds (including not only pesticides but also other contaminants), with the main aim to enable target as well as post-target and non-target screening to identify unexpected or unknown substances [10,11,18–21]. The utility of such approach can be found in domains of foodomics, fingerprinting techniques and environmental forensics [22]. It is commonly recognized that such approaches cannot reach equal quantitative accuracy as that of triple quadrupole [3,6,18,21]. However, the recent advances and increasing sophistication of MS products expand its capabilities further, providing higher sensitivity, lower costs, increasing sample throughput and improving the dynamic range and linearity.

The Orbitrap™ mass analyser, developed by Makarov ten years ago and commercially introduced in 2005, implements the principles of Fourier transform (FT) through an electrostatic axially harmonic orbital trapping technique to yield high resolution mass

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0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.12.082

spectra [23]. The standalone instrument, which provides high mass resolution (>15,000 FMWH) and high mass accuracy (<2 ppm) but without mass selection, is already successfully used in routine for determining pesticide residues [12,21,24–29]. The combination of a low resolution linear ion tap with the high resolution Orbitrap analyzer (LTQ Orbitrap) has also been used occasionally for determining triazines in rice [8] and acidic herbicides in wastewater effluents [30], demonstrating to be highly versatile and effective because not only all modes of LTQ remain available but also are complemented by the ability to analyze ions in the orbitrap [8,30,31]. The current generation of LTQ-Orbitrap instruments has significant features an S-lens with up to 10 fold improved ion transmission for the atmosphere, a dual linear ion trap, and a more efficient higher energy collisional dissociation (HCD) cell interfaced directly to the C-trap (schematized in Supplementary material Fig. S1). These instruments are capable of much higher scan speed resolution compared with the old ones and offer a range of fragmentation modes depending on the analytical problem. To the best of our knowledge, new LTQ-Orbitraps have only been applied in combination with direct analysis in real time (DART) to directly identify xenobiotics in fruits peel [32]. However, combination DART-Orbitrap does not allow fully exploitation of MS/MS possibilities. Recently, the combination of a quadrupole with an orbitrap (Q-Orbitrap), which also showed high ion currents because of the S-lens, and fast high energy collision induced dissociation fragmentation, was also successfully applied to determine pesticide residues in fruits and vegetables [33]. Although sensitivity of the Q-Orbitrap is not limiting, it only can perform HCD fragmentation, making its fragmentation less versatile than in the LTQ-Orbitrap.

Reported here is the first application of one of the last generation LTQ Orbitrap-MS for the screening and quantification of a large number of pesticide residues and the characterization of other several contaminants in a number of environmental and food samples. The present work implemented a Velos Orbitrap mass spectrometer in the routine screening of pesticides. The LC–HRMS method was developed and optimized for 54 pesticides in a complex environmental and food matrices including water, fruits, fish, meat, and honeybees. Data dependent MS^2 and MS^3 acquisition confirmation based on collision induced dissociation (CID) was used. Furthermore, in the last part of this study, the capabilities to identify other non-target contaminants that could be present in the analyzed food and environmental matrices were explored.

2. Materials and methods

2.1. Reagents and standard solutions

High purity (98–99.9%) standards of 54 selected pesticides (Table 1) were obtained from Sigma–Aldrich (Steinheim, Germany) or Riel-de-Haen AG (Seelze, Germany). Individual standard solutions were prepared in methanol at a concentration of 1000 mg L^{-1}. The standard mixture was prepared by mixing the appropriate amounts of the individual standard solutions and diluting with methanol to a final concentration of 5 μg mL^{-1}. Working solutions were prepared daily by diluting the standard mixture with acetonitrile or methanol. All solutions were stored in amber glass bottles at 4 °C in the dark.

Formic acid were purchased from Sigma–Aldrich, and dichloromethane and methanol (gradient grade for liquid chromatography), were obtained from Merck (Darmstadt, Germany). High purity water was prepared using a Milli-Q water purification system (Millipore, Milford, MA, USA). Dichloromethane–methanol (50:50, v/v) was used to elute the pesticides from the Oasis HLB SPE cartridge (200 mg sorbent/6 mL cartridge, Waters). Acetonitrile (CH_3CN, ≥99.9%) was from Honeywell Burdik & Jackson (Muskegon, USA), magnesium sulphate ($MgSO_4$, 99.5%) from Alfa Aesar GmbH & CoKG (Karlstuhe, Germany), sodium chlorate (NaCl, 95.5%), trisodium citrate dehydrate ($Na_3C_6H_5O_7{\cdot}2H_2O$, 99.5%) and disodium hydrogen citrate sesquihydrate [$HOC(COOH)(CH_2COONa)_2{\cdot}1.5\ H_2O$, >99%] from Merck KGaA (Darmstadt, Germany), primary secondary amine (PSA), C_{18} and graphitized black carbon (GBC) from Análisis Vinícos S.L. (Tomelloso, Spain).

2.2. Samples

For fruits, blank samples were obtained from an organic agricultural cooperative that ensure pesticide free samples. Water, fish and bee samples were obtained for previous years processed samples that were already analyzed for a number of compounds. These blank samples were used for validation purposes. After extraction, these samples were also checked in the UHPLC–Orbitrap-MS in order to evaluate possible contaminants. These samples present several peaks. However, all of them were attributed to the system since they are also present in solvents and procedural blanks. Some ubiquitous contaminants were at *m/z* 279.15919 (identified as the plasticizer dibuthylphthalate) and *m/z* 339.34182 (identified as Erucamide). Both of them have been widely reported in LC–MS [34]. All these background was subtracted in the surveyed samples as well as in the standards and the spiked samples.

A short survey was carried out in 8 fruits (2 apples, 2 lemons, 2 oranges and 2 tomatoes) taken from a local market, 4 fish samples—2 Carp (*Cyprinus carpus*) and 2 European catfish (*Silurus glanis*)—, 5 waste waters and 3 sludges taken from the Ebro River (in Spain) and 3 honey bee (*Apis melifera*) samples provided by a local beekeeping cooperative. These samples were collected, transported to the laboratory and preserved following standard procedures that guarantee their integrity until the analysis.

2.3. UHPLC/ESI LTQ-Orbitrap parameters

The UHPLC–LTQ-Orbitrap system consisted of a Transcend LX-2 UHPLC (part of the TurboFlow TLX-2 that was not used in the present study) with Allegro quaternary pumps (Thermo Fisher Scientific, Basel, Switzerland) coupled with a LTQ Orbitrap Velos mass spectrometer (ThermoFisher Scientific) (Supplementary material Fig. S1). Instrument control was through Tune 2.6.0 and Xcalibur 2.2 programmes. The UHPLC column utilized was a Kinetex 1.7 μm XB-C_{18} 100 Å (5 mm × 2.10 mm) equipped with SecurityGuard ULTRA Cartridges UHPLC C_{18} for 2.1 mm ID Columns both from Phenomenex (Cheshire, UK). UHPLC mobile phase B was 0.1% formic acid in methanol, and mobile phase A 0.1% of formic acid in water. Separation was carried out in 12 min at a flow rate of 0.3 mL min^{-1} under the following conditions: 0 min, 30% B; 10 min, 95% B; 12 min, 98% B. Column oven temperature was set at 30 °C, and autosampler temperature was set at 20 °C. Injection volume was 5 μL, and total run time was 15 min.

Ion source was equipped with a heated electrospray ionization (HESI) probe and was tuned and calibrated using the calibration solution once a week. The capillary temperature was 275 °C, the source voltage 3 kV and the S-lens RF level was fixed at 67%. Accurate mass spectra were recorded from 50 to 500 *m/z*. The external mass calibration of the orbitrap was performed once a week to ensure a working mass accuracy < 3 ppm. According to the manufacturer's directions, a mixture of caffeine, MRFA peptide, and Ultramark for positive ionization mode or a mixture of MRFA peptide, Ultramark, SDS, and sodium taurocholate for negative ionization mode were used. The mass resolution was tuned to 30,000 FWHM at *m/z* 400 with the standard 384 ms transient and an automatic gain control (AGC) of 1×10^6 with a maximum accumulation time in the C-trap of 100 ms, which achieves