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Large pesticide multiresidue screening method by liquid chromatography-Orbitrap mass spectrometry in full scan mode applied to fruit and vegetables

Łukasz Rajski, María del Mar Gómez-Ramos, Amadeo R. Fernández-Alba*

European Union Reference Laboratory for Pesticide Residues in Fruit & Vegetables. University of Almeria, Agrifood Campus of International Excellence (ceiA3) Department of Hydrogeology and Analytical Chemistry, Ctra. Sacramento S/N°, La Cañada de San Urbano, 04120, Almería, Spain

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ABSTRACT

The present work is focused on evaluating the main operational parameters for multiresidue screening of an Orbitrap mass spectrometer for pesticide residue analysis in fruits and vegetables. Operational parameters such as resolution, software for the automatic detection, mass tolerance and retention time extraction window, along with the analytical performance, were evaluated in an updated UHPLC-Orbitrap-mass spectrometer working in full scan mode. The evaluation was performed using QuEChERS extracts of tomato, pepper, orange and green tea. The extracts were spiked with 170 selected pesticides at four concentration levels (10 μg/kg, 50 μg/kg, 100 μg/kg and 500 μg/kg). Extracts were diluted 5 fold before injection. Three different resolution settings (17,500, 35,000 and 70,000) were evaluated at various concentration levels. At 10 μg/kg, using a resolution of 17,500 and 5 ppm of mass tolerance, the detected pesticide rates were from 91% in tomato, to 83% in green tea. These percentages increased at higher resolution values. A resolution of 70,000 was adequate for such analysis even when a small percentage of false detect at low concentration was obtained. The rates of detected compounds increased and were from 98% in tomato to 88% in green tea. Mass tolerance of 5 ppm was the most adequate for screening purposes. The observed false negative detects were mainly a consequence of a lack of compound sensitivity exacerbated by ion suppression effects in the experimental conditions applied. With reporting limits of 10 μg/kg, reproducibility improved with resolution levels of 35,000 or higher. Linearity was investigated in the 2–100 ng/mL (equivalent to 10–500 μg/kg in the sample) range. Particularly good automatic screening effectiveness was obtained using the selected settings in the analysis of real samples where no false negatives detects and 5% of false positives detects were obtained.

1. Introduction

Triple quadrupole (QqQ) based mass spectrometers coupled to gas and liquid chromatographs are the most important analysers used for multiresidue pesticide analysis in food. This is because of their excellent quantitation and identification properties for a group of target compounds [1]. However, these instruments have certain limitations: they require acquisition parameter optimisation for each analysed compound, the number of analysed compounds is limited, only compounds from a target list can be detected and retrospective data analysis is impossible. Furthermore, in matrices with a high number of coextractives, called "difficult" matrices, identification criteria based on two transitions, are not always enough for an unambiguous positive detection [2]. This fact can originate, apart from human errors, from very low transition abundance or from identical or close abundance ratio transitions from the matrix. In those cases the application of an orthogonal mass spectrometry technique such as high resolution mass spectrometry (HRMS) is required. The application of HRMS offers new possibilities and minimizes the limitations mentioned above [3]. High resolution mass spectrometers operating in full scan mode offer a significant increase of the analysis selectivity and can detect an unlimited number of compounds. Optimization of analysis parameters is much less laborious and retrospective analysis is possible [4]. Moreover, with HRMS, it is possible to identify analytes without previous injection of the corresponding analytical standards [5].

Kaufmann et al. compared the HRMS instrument (a single-stage Orbitrap) with a triple quadrupole mass spectrometer on a large number of pesticides. Considering precision, accuracy,

* Corresponding author. Tel.: +34 950 015 034.
E-mail address: amadeo@ual.es (A.R. Fernández-Alba).

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sensitivity, dynamic range and selectivity, the authors concluded that HRMS could adequately compete with QqQ in the field of quantitative analysis [1]. A similar investigation was conducted by Alder et al. Their study was wider and included 500 compounds in five matrices. However, it was limited to detection only and no quantitation was carried out. In the opinion of the authors, HRMS could ensure sufficient sensitivity for pesticide detection in the matrices examined [5]. A resolution of 50,000 (FWHM at m/z 200) was able to guarantee selectivity equal to or even better than MS/MS with a satisfactory number of points per peak [6].

The Orbitrap mass analyser was described for the first time in the year 2000 by Makarov [7]. Ions are introduced into the Orbitrap in small packets. A strong electrical field inside the analyser initiates axial oscillation. Ions oscillate harmonically with a period proportional to $(m/z)^{1/2}$ and produce an image current on split outer electrodes. Subsequently, the signal obtained is converted from time-domain into a mass spectrum by Fourier transformation [8].

The Orbitrap was introduced onto the market in 2005. Now spectrometers equipped with Orbitrap analysers are available in diverse configurations – as a single-stage mass spectrometer and as a hybrid instrument (with a quadrupole mass filter or a linear ion trap).

In its early days, the Orbitrap was considered as a tool mainly for proteomic and metabolomic applications [3]. However, over recent years, interest has been steadily growing in the application of Orbitrap to environmental samples and to the field of food safety. Examples of single-stage Orbitrap applications, as well as hybrid instruments, can be found in the literature. Kauffman et al. analysed over 100 veterinary drugs in food matrices (muscle, kidney, liver, fish and honey). In addition to validation, the authors also compared results obtained with resolutions of 50,000 (Orbitrap, FWHM at m/z 200) and 12,000 (TOF) [6]. Mol et al. investigated the single-stage Orbitrap as a tool for pesticide screening in fruits and vegetables. The authors analysed over 500 pesticides in 21 commodities with a resolution of 50,000 (m/z 200). One of the main conclusions of the work was that identification based on retention time and only one diagnostic ion may result in a considerable number of false positives [9]. In the case of plant toxins analysis in food and feed, using a resolution of 100,000 (FWHM at m/z 200), it was possible to reduce mass error below 2 ppm for most of the analytes [10]. Single-stage Orbitrap was also successfully employed for the analysis of veterinary drugs in animal urine [11] as well as plant and fungal metabolites in cereals and animal feed [12].

With the Orbitrap resolution is inversely proportional to square root m/z [13]. In the rest of this paper, all resolution values are referred to m/z 200.

With instrument upgrades and the introduction of hybrid instruments offering the possibility of performing full scan MS and MS/MS simultaneously, or sequential experiments, many of drawbacks have disappeared. Full scan and MS/MS analysis can be carried out separately as well as in one run allowing the verification of results. Hybrid instruments were applied to synthetic hormones in animal urine [14], acidic contaminants in wastewater effluents [15] and pesticides in fruits and vegetables [16].

In our opinion, as is described in the present work, Orbitrap mass analyzers, and HRMS in general, are very flexible and complementary instruments to triple quads as they are able to offer similar quantitative performance. Combining both in routine food laboratories can consistently cover the main legal requirements with regard to pesticides and, at the same time, enlarge the analytical scope in a most cost effective way, avoiding false positives and negatives, and, additionally, allowing a retrospective evaluation. Nonetheless, there is a lack of information regarding the settings, operational requirements and detection capabilities of the last generation of HRMS instruments for screening analysis, considering the large number of pesticide residues that can be present, combined with the difficulties that the different matrices might present.

Even when screening methods can be focused solely on aspects of identification, in the field of residues analysis, we view such identification, to some extent, as inseparable from the properties of quantification – given that only compounds detected above a certain reporting level can be considered positive findings. For this reason, both aspects are considered here. The current work is focused on a detailed study of the main settings of this type of instrumentation and the analytical performance that can be achieved applying it to pesticide residues analysis in fruits and vegetables.

2. Material and methods

2.1. Reagents and materials

All high purity pesticide standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and Riedel-de Haën (Selze, Germany) and were stored at −30 °C. Individual pesticide stock solutions (1000–2000 mg/L) were prepared in acetonitrile and ethyl acetate and were stored in amber screw-capped glass vials in the dark at −20 °C. Individual standard solutions, used for the optimization, along with standard-mix solutions, used for the calibration, were prepared from the stock standards.

Water was obtained from Fisher Scientific (Fair Lawn, NJ, USA), methanol from Fluka Analytical (Steinheim, Germany). Ammonium formate and formic acid were purchased from Sigma–Aldrich (Steinheim, Germany).

Pierce LTQ Velos ESI Positive Ion Calibration Solution and Pierce LTQ Velos ESI Negative Ion Calibration Solution were provided by Thermo Fisher Scientific (Waltham, MA, USA).

2.2. LC-MS analysis

2.2.1. LC-QExactive

For the LC separation, UHPLC Dionex Ultimate 3000 (Thermo Scientific, San Jose, USA) was used. Mobile phase A was 98% water and 2% methanol whereas mobile phase B was 98% methanol and 2% water; both mobile phases contained 5 mM of ammonium formate and 0,1% formic acid. Separation was carried out on a Thermo Scientific Accucore aQ C18 column. The length, diameter and particle size were 150 mm, 2.1 mm and 2.6 μm, respectively. The column was thermostatted at 25 °C. Three minutes before injection, the column was equilibrated with 100% of mobile phase A. From 0 to 4 min, the amount of mobile phase B increased to 20%, from 4 to 5.5 min to 40%, and from 5.5 to 10 min to 100%. 100% of B was maintained until 13 minutes. Following this, the mobile phase was changed to 100% A and maintained over 5 minutes for reequlibration. The injection volume was 10 μL. The autosampler was thermostatted at 10 °C.

A QExactive (Thermo Scientific, Bremen, Germany) mass spectrometer was equipped with Heated Electrospray Ionization Source (HESI II). The HESI parameters in positive polarity were as follows: sheath gas flow rate: 40; auxiliary gas flow rate: 5; sweep gas flow rate: 1; spray voltage: 3.00 kV; capillary temperature: 280 °C; S-lens RF level: 55.0; heater temperature: 350 °C. To select optimal capillary temperature values of 180 °C, 280 °C and 380 °C were tested. Fig. 1 presents effects of capillary temperature on sensitivity. The negative polarity parameters were the same as in the positive. QExactive worked in full scan mode. The AGC target was set to 1e6, the maximum IT was set to 200 ms. The scan covered masses in the m/z 100–800 range. Experiments were carried out at three resolutions: 17,500, 35,000 and 70,000 (FWHM at m/z 200). The external calibration of the Orbitrap was carried out daily. For the calibration, a mixture containing n-butylamine, caffeine, Ultramark 1621