Journal of Chromatography A, 1374 (2014) 66–76

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Comprehensive analytical strategy for biomonitoring of pesticides in urine by liquid chromatography–orbitrap high resolution mass spectrometry

M. Roca[a], N. Leon[a], A. Pastor[b], V. Yusà[a,b,*]

[a] Laboratory of Public Health of Valencia (LSPV-FISABIO), 21 Avenida Catalunya, 46020 Valencia, Spain
[b] Department of Analytical Chemistry, Chemistry University of Valencia, 50 Doctor Moliner, 46100 Burjassot, Spain

ARTICLE INFO

Article history:
Received 4 August 2014
Received in revised form 5 November 2014
Accepted 6 November 2014
Available online 25 November 2014

Keywords:
High-resolution mass spectrometry
Pesticides
Urine
Biomonitoring
Orbitrap

ABSTRACT

In this study we propose an analytical strategy that combines a target approach for the quantitative analysis of contemporary pesticide metabolites with a comprehensive post-target screening for the identification of biomarkers of exposure to environmental contaminants in urine using liquid chromatography coupled to high-resolution mass spectrometry (LC–HRMS). The quantitative method for the target analysis of 29 urinary metabolites of organophosphate (OP) insecticides, synthetic pyrethroids, herbicides and fungicides was validated after a previous statistical optimization of the main factors governing the ion source ionization and a fragmentation study using the high energy collision dissociation (HCD) cell. The full scan accurate mass data were acquired with a resolving power of 50,000 FWHM (scan speed, 2 Hz), in both ESI+ and ESI− modes, and with and without HCD-fragmentation. The method – LOQ was lower than 3.2 $\mu g\,L^{-1}$ for the majority of the analytes. For post-target screening a customized theoretical database was built, for the identification of 60 metabolites including pesticides, PAHs, phenols, and other metabolites of environmental pollutants. For identification purposes, accurate exact mass with less than 5 ppm, and diagnostic ions including isotopes and/or fragments were used. The analytical strategy was applied to 20 urine sample collected from children living in Valencia Region. Eleven target metabolites were detected with concentrations ranging from 1.18 to 131 $\mu g\,L^{-1}$. Likewise, several compounds were tentatively identified in the post-target analysis belonging to the families of phthalates, phenols and parabenes. The proposed strategy is suitable for the determination of target pesticide biomarkers in urine in the framework of biomonitoring studies, and appropriate for the identification of other non-target metabolites.

1. Introduction

In recent years the health effects associated with the application of contemporary pesticides in agricultural and residential areas has become a great public concern, as literature shows different associations between non-persistent pesticide exposure and several human health outcomes [1–3]. Consequently, a comprehensive risk assessment is mandatory to assess population exposure to these chemicals and develop adequate actions for reducing it. The European Environmental and Health Strategy and its corresponding Action Plan 2004–2010 [4] encouraged the adoption of human biomonitoring [5] studies in Europe, as an effective and necessary tool for assessing human exposure to chemicals in different population groups, mainly children [5]. Thus, these biomonitoring programs require appropriate analytical methods [6].

Organophosphate (OP) insecticides together with a variety of herbicides including phenoxyacetic acids, chloroacetanilides and triazines, synthetic pyrethroids, and several carbamate fungicides are among the most widely used contemporary pesticides for agricultural, domestic and gardening purposes in the USA and Europe [7]. These compounds are considered as non-persistent pesticides due to their short environmental half-lives [8,9]. Furthermore, these chemicals tend not to bio-accumulate and they are typically metabolized and excreted in urine within 24–48 h as a mercapturate detoxification product, as free metabolites, and/or as glucoronide or sulfate-bound metabolites [10].

Biomarkers of pesticide exposure are present in the general population at concentrations of few $\mu g\,L^{-1}$, and consequently

* Corresponding author at: Laboratory of Public Health of Valencia (LSPV-FISABIO), 21 Avenida Catalunya, 46020 Valencia, Spain. Tel.: +34 961925865; fax: +34 961925888.
E-mail address: yusa_vic@gva.es (V. Yusà).

http://dx.doi.org/10.1016/j.chroma.2014.11.010
0021-9673/

their determination requires sensitive and selective analytical methods. Likewise, the use of high-throughput methods seem to be necessary for the analysis of the large number of samples usually required in HBM programmes [11–13]. Nowadays, liquid chromatography–tandem mass spectrometry (LC–MS/MS) has become the most frequently used analytical technique for the biomonitoring of pesticides in urine, with several methods having been published in the last four years [14–17]. These target methods follow the analytical strategy prevailing so far in this area, consisting of the development of very sensitive methods with a reduced analytical scope (6–12 analytes) along with the use of specific and intensive sample treatment, mainly using solid-phase extraction.

Although LC–MS/MS presents a high sensitivity and selectivity, it requires an extensive method set-up, mainly for large-scope multi-residue methods, and presents limitations with regard to the number of compounds with different physico-chemical properties that can be analyzed in one run. Moreover, a retrospective analysis of the sample cannot be conducted for the post-target analysis of other relevant compounds not included in the initial scope. For pesticide determination in food and environmental analysis LC coupled to high-resolution mass spectrometers (HRMS) has increasingly become more common, having many papers been published in the last four years [18,19]. The use of HRMS is largely driven by the advantages of using the full-scan acquisition mode with high sensitivity, combined with high-resolving power (>50,000 FWHM) and accurate mass measurement (1–5 ppm) [18]. This analytical technique also allows the development of comprehensive strategies combining the quantitative target analysis of priority analytes with the post-run target or non-target analysis, including the retrospective analysis for searching new compounds [20–22].

The emerging trend towards accurate-mass and full-scan comprehensive methods needs to be supported by the development of generic sample preparation methodologies, capable of extracting a large number of substances with different physico-chemical properties, both for target and post-target analysis purposes [23]. Nowadays, the QuEChERS (standing for Quick, Easy, Cheap, Effective, Rugged and Safe) procedure has been widely used in the pesticide field [24,25] and veterinary residue analysis in different matrices [26]. Nevertheless this procedure has not been used before for the analysis of pesticides and/or their metabolites in human urine samples.

In the present study we have developed an analytical strategy that combines the quantitative target analysis of 29 relevant biomarkers of exposure to contemporary pesticides, with the post-target screening (identification) of biomarkers of exposure to other environmental pollutants in urine, using a generic extraction method based on QuEChERS and LC–HRMS. To our knowledge, there is no previously reported work for the analysis of pesticide biomarkers in urine using this approach.

2. Experimental

2.1. Chemicals and reagents

All solvents used were specific for pesticide residue analysis and of analytical grade. Acetonitrile and methanol were supplied by Scharlab (Barcelona, Spain). Acetic acid (purity 98–100%), β-glucuronidasa aryl sulfatase enzyme, and anhydrous sodium acetate were obtained from Merck (KGaA, Darmstadt, Germany). Deionized water was organically and biologically purified by using a Milli-Q Ultrapure System (Millipore, Darmstadt, Germany). QuEChERS EN extraction kits, containing 4 g $MgSO_4$; 1 g NaCl, 1 g NaCitrate; 0.5 g disodium citrate sesquihydrate, were obtained from Agilent Technologies (Madrid, Spain).

2.2. Standards and stock solutions

Certified commercial standards were of high purity and purchased from Dr. Ehrenstorfer (Augsburg, Germany), Sigma–Aldrich (Barcelona, Spain), Cerilliant-Certificated Reference Materials (Texas, USA), and Cambridge Isotope Laboratories (Massachusetts, USA). In Table 1 the metabolites and Internal standard (IS) used in the target quantitative analysis are presented. Stock standard solutions of individual compounds (with concentrations ranging between 20 and 500 mg L^{-1}) were prepared in acetonitrile by weighing powder and stored at −20 °C. Multi-analyte intermediate standard solutions were prepared by diluting the individual stock solutions with acetonitrile and used for preparing working mixed-standard solutions in acetonitrile: water (10:90, v/v). The concentration of the analytes in working solutions ranged from 1000 to 5000 ng mL^{-1} depending on the compound. A working solution of 1000 ng mL^{-1} was also prepared containing internal standards.

2.3. Sample preparation

After the homogenization of the whole sample, 5 mL of urine were mixed into a 15 mL tube with 1 mL of 0.2 M acetate buffer (3.1 mL of glacial acetic acid and 9.7 g of sodium acetate diluted in 1 L of deionized water), 10 μL of β-glucuronidase aryl sulfatase enzyme (to hydrolyze possible glucuronide- or sulfate-conjugated metabolites), and the internal standard solution. The samples were incubated overnight at 37 °C (10–17 h).

After the enzymatic hydrolysis, a simplified QuEChERS procedure was employed to extract metabolites from urine samples. Briefly, 10 mL of acetonitrile, and a pouch of QuEChERS EN extraction salt packet were added to the hydrolysed sample in a 50 mL polypropylene tube. The mixture was strongly shaken and centrifuged during 10 min at 3500 rpm. The acetonitrile layer obtained was immediately transferred into a 15 mL tube and evaporated to dryness in a water bath at 37 °C under a stream of nitrogen. The dry residue was then dissolved in 200 μL of methanol: water (10/90, v/v) containing 0.1% of acetic acid, placed into a Millipore 0.2 μm Eppendorf and ultra-centrifuged (11,000 rpm, 3 min and 10 °C). The final extract was transferred into an injection vial and analyzed on the UHPLC–HRMS system.

2.4. UHPLC–HRMS analysis

Chromatographic separation was performed on an Accela liquid chromatography UHPLC system from ThermoFisher Scientific (Bremen, Germany). The flow rate used was 400 μL min^{-1} and the injection volume was 10 μL. Separations were performed using a binary gradient. Various mobile phases and chromatographic columns were tested in order to achieve a good separation and peak shape for all the 29 target metabolites. Taking into consideration the generic aspects of the analytical procedure, two C18-based columns (AQ Hypersil Gold and Hypersyl Gold, both of 100 × 2.1 mm, 1.9 μm), from ThermoFisher, used in previous muli-residue studies, as well as common solvents (methanol and acetonitrile) and modifiers (formic and acetic acid) were tested [27].

The analysis started with 95% mobile phase A. After 1 min, this percentage was linearly decreased down to 45% within 5 min. After that, solvent A decreased quickly to 0% in 0.5 min and maintained for 1.5 min. The composition was increased to initial conditions in 0.5 min, followed by a re-equilibration time of 12.5 min. The total run time was 20 min. Data acquisition was performed by the Thermo Scientific Trace Finder 3.1 software.

Mass analysis was performed on the Orbitrap mass spectrometer Exactive™ analyser (Thermo Scientific, Bremen, Germany).