Science of the Total Environment 622–623 (2018) 526–533

Contents lists available at ScienceDirect

Science of the Total Environment

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

Analysis of metabolites of organophosphate and pyrethroid pesticides in human urine from urban and agricultural populations (Catalonia and Galicia)

Mercè Garí [a,b], Yolanda González-Quinteiro [b], Natalia Bravo [b], Joan O. Grimalt [b,*]

[a] Department of Earth and Ocean Dynamics, University of Barcelona (UB), Barcelona, Catalonia, Spain
[b] Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDÆA-CSIC), Jordi Girona, 18, 08034 Barcelona, Catalonia, Spain

HIGHLIGHTS

- An UPLC-MS/MS method for analysis of urine organophosphate metabolites was developed.
- An UPLC-MS/MS method for analysis of human urine pyrethroid metabolites was developed.
- The use of synthetic urine afforded calibration straight lines with lower detection limits.
- Detection limits were in the range of 14–69 pg/ml.
- Organophosphate concentrations in farmworkers was twofold than in urban populations.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:
Received 30 September 2017
Received in revised form 29 November 2017
Accepted 30 November 2017
Available online xxxx

Keywords:
Ultra-performance liquid chromatography
Tandem mass spectrometry
Urinary metabolites
Organophosphate pesticides
Pyrethroids

ABSTRACT

Isotope dilution solid phase extraction UPLC-MS/MS has been used to develop a robust and rapid methodology for the determination of eight specific metabolites of organophosphate and pyrethroid pesticides in human urine. The use of methanol:acetone (25:75 *v*/v) affords an improvement in extraction efficiency in comparison to these individual solvents. The use of synthetic urine improves selectivity and limits of detection for the calibration straight lines. The method provides detection limits of 14–69 pg/ml and 18–19 pg/ml for the organophosphate and pyrethroid metabolites, respectively. Urine analyses of these metabolites in urban non-occupationally exposed individuals and farm workers shows that ingestion of these pesticides occurred in both populations. The concentrations of organophosphate pesticide metabolites in the latter were twofold than those from non-exposed populations.

1. Introduction

Organophosphate (OP) and pyrethroid (PYR) pesticides are commonly used in agriculture as well as for domestic and gardening use. They eliminate insects because of their strong potential to disrupt the brain and nervous system of these organisms. Unfortunately, this neurotoxic effect is not selective enough as to avoid damage to other non-target species, including humans (Barr, 2008). There is growing public concern on pesticide use not only for the negative impacts on wildlife and the environment but also for the potential adverse health effects on humans. OP and PYR pesticide exposure has been related to several

* Corresponding author.
E-mail address: joan.grimalt@idaea.csic.es (J.O. Grimalt).

https://doi.org/10.1016/j.scitotenv.2017.11.355
0048-9697/

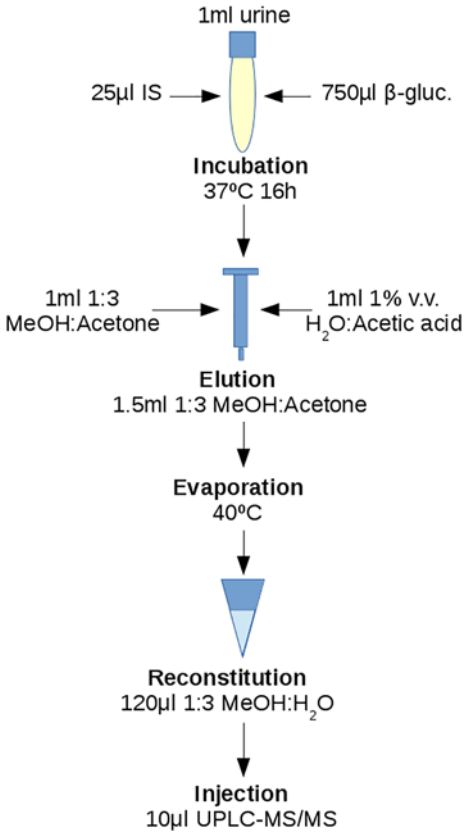

Fig. 1. Graphical representation of the extraction procedure.

health effects, including respiratory, digestive, reproductive and neurological problems, among others (Ye et al., 2013; Arcury et al., 2016; Llop et al., 2017).

Once in the human body, OP and PYR pesticides are typically metabolized and excreted in urine within 4–48 h after exposure, depending on the compound (Egeghy et al., 2011). Organophosphates are metabolized into dialkyl phosphates (DAPs) and specific compounds, including 3,5,6-trichloro-2-pyridinol (TCPY, the metabolite of chlorpyriphos), 4-nitrophenol (PNP, metabolite of parathion), malathion dicarboxylic acid (MDA, metabolite of malathion), 3-chloro-4-methyl-7-hydroxycoumarin (CMHC, metabolite of coumaphos), 2-isopropyl-6-methyl-4-pyrimidiol (IMPY, metabolite of diazinon) and 2-diethylamino-6-methylpyrimidin-4-ol (DEAMPY, metabolite of pirimiphos). For the most common pyrethroids, which include permethrin, cypermethrin, deltamethrin and esfenvalerate, all these pesticides are metabolized into one single compound, 3-phenoxybenzoic acid (3-PBA). Cyfluthrin pesticide is metabolized into 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA). Therefore, 3-PBA and 4-F-3-PBA can be used as a biomarker of the most common PYR pesticides (Barr, 2008; Ueyama et al., 2010; Egeghy et al., 2011).

Urine analysis is the simplest and least intrusive method for assessing human exposure to the aforementioned non-persistent pesticides. Previously published methods for the analysis of specific metabolites of OP and PYR pesticides in urine are based on both gas and liquid chromatography, and mainly using mass spectrometry techniques (Koureas et al., 2012). The concentrations of metabolites of these compounds in urine reflect the exposure levels of the individuals (Barr, 2008). Farmworkers and rural populations are in principle potentially more exposed to these pesticides than general populations (Arcury et al., 2007). However, the low concentrations of these metabolites in urine, currently in the order of ng/ml, and the large numbers of samples needed for epidemiological studies require robust, cheap and efficient analytical methodologies (Barr, 2008). In this context, the limits of detection (LD) are critical to discriminate for the presence of the analytes and for feasibility of study of high numbers of individuals and possible health effects (Currie, 1997; Koch et al., 2001; Ye et al., 2013).

In many of these epidemiological or population toxicity studies these limits are not only considered as analytical parameters but as reference for classification between individuals (Ueyama et al., 2010; Davis et al., 2013; Olsson et al., 2004; Barr et al., 2010; Koureas et al., 2012; Roca et al., 2014a, 2014b). Fulfilling the requirements for the use of detection limits following this approach requires extraction procedures adapted to the most representative conditions of real samples (Garí and Grimalt, 2010). In this context, interferences from human urine may increase limits of detection and distort calibration straight lines. Thus, the developed methodology must consider matrix effects and their variability. The use of synthetic urine instead of urine dilution may provide robust procedures to fulfill these requirements.

Accordingly, a new analytical methodology for the quantification of OP and PYR urinary specific metabolites has been developed in the present study. This method takes into account the variability of concentrations found in human urines from both general and highly exposed populations from rural or agricultural sites. The method is based on ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) and allows the quantification of eight biomarkers of several of these pesticides using only one ml of urine. It provides high precision and accuracy, and low detection limits to analyze these pesticide metabolites both in professionally exposed farmers and non-exposed general population.

2. Materials and methods

2.1. Standards, solvents and reagents

Standards of IMPY and TCPY were purchased from Sigma-Aldrich (Madrid, Spain), PNP from Supelco (Madrid, Spain), CMHC from Acros Organics (Geel, Belgium), DEAMPY, MDA, 3-PBA and 4-F-3-PBA from Dr. Ehrenstoffer (Augsburg, Germany). The isotopically-labeled

Table 1
Instrumental analytical data of the organophosphate and pyrethroid pesticide metabolites considered in the present study.

Acronym	Analyte	Q-SRM[a]	C-SRM[b]	Ion ratio	Collision energy	Cone voltage	Retention time
DEAMPY[c]	2-diethylamino-6-methyl pyrimidin-4-ol	182–154	182–84	1.3	20	40	4.65
IMPY[c]	2-isopropyl-6-methyl-4-pyrimidiol	153–84	153–70	1.9	20	40	5.05
MDA[d]	Malathion dicarboxylic acid	273–141	273–157	2.6	8	25	8.65
PNP[d]	4-nitrophenol	138–108	138–92	8.2	20	45	8.66
CMHC[d]	3-chloro-4-methyl-7-hydroxicoumarin	209–145	209–117	3.2	25	20	9.70
TCPY[d]	3,5,6-trichloro-2-pyridinol	196–196	198–198	1.0	7	10	11.28
3-PBA[d]	3-phenoxybenzoic acid	213–93	213–169	1.6	20	30	12.87
4-F-3-PBA[d]	4-fluoro-3-phenoxybenzoic acid	231–187	231–93	1.3	15	25	13.04

[a] Q-SRM: Quantification Selected Reaction Monitoring.
[b] C-SRM: Confirmation Selected Reaction Monitoring.
[c] Positive ion mode.
[d] Negative ion mode.