Journal of Chromatography A, 1303 (2013) 48–53

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

Analysis of organophosphate flame retardant diester metabolites in human urine by liquid chromatography electrospray ionisation tandem mass spectrometry

Nele Van den Eede[a], Hugo Neels[a], Philippe G. Jorens[b], Adrian Covaci[a,*]

[a] Toxicological Center, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[b] Clinical Pharmacology/Toxicology, University of Antwerp, Antwerp University Hospital, Wilrijkstraat 10, 2630 Edegem, Belgium

ARTICLE INFO

Article history:
Received 4 April 2013
Received in revised form 7 June 2013
Accepted 19 June 2013
Available online 26 June 2013

Keywords:
Dialkyl phosphates
Diphenyl phosphate
Phosphorus flame retardants
PFR
Metabolites
Urine

ABSTRACT

A new analytical method was developed for the determination of dialkyl and diaryl phosphates (DAPs), which are metabolites of organophosphate triesters (PFRs), in human urine. Target DAPs included dibutyl phosphate (DBP), diphenyl phosphate (DPHP), bis(2-butoxyethyl) phosphate (BBOEP), bis(2-chloroethyl) phosphate (BCEP), bis(1-chloro-2-propyl) phosphate (BCPP), and bis(1,3-dichloro-2-propyl) phosphate (BDCIPP). Sample preparation was based on solid phase extraction using a weak anion exchange sorbent (Oasis WAX). Although several instrumental techniques have been tested, best results were obtained with reversed phase liquid chromatography–negative electrospray ionisation tandem mass spectrometry (LC–ESI-MS/MS) taking the total analysis time into account. Method accuracy at 3 ng/mL in pooled urine ranged between 69 and 119% (recovery), while inter-day imprecision (as relative standard deviation) was <31%. The performance of the LC–MS/MS method was compared to a method based on gas chromatography–electron impact tandem mass spectrometry (GC–MS/MS) and a good correlation (Pearson $r = 0.82$, $p < 0.01$) between the results of these two methods was obtained for DPHP. LC–MS/MS analysis was more suitable for DPHP and BBOEP with respective method limits of quantification (mLOQ) of 0.3 and 0.15 ng/mL. In contrast, GC–MS/MS had a better sensitivity for BCEP, BCIPP, and BDCIPP, their respective mLOQs being 0.1, 0.06, 0.02 ng/mL, compared to 1.2, 3.7, and 0.5 ng/mL by LC–MS/MS. A set of urine samples from volunteers was analysed, in which DPHP was the major DAP metabolite. A significant increase of DPHP levels was observed in the group of smokers (geometric mean of 1.55 ng/mL) compared to the non-smokers (geometric mean of 0.88 ng/mL). Metabolic transformation of triphenyl phosphate to DPHP by metabolic enzymes induced in smokers could be an explanation for this observation.

1. Introduction

Organophosphate triesters are used as additive flame retardants (PFRs) and plasticisers in polymers, paints, synthetic rubber, varnishes, lacquers, and hydraulic fluids [1,2]. Since the restriction in the use of the flame-retarding polybrominated diphenyl ethers (PBDEs), the consumption of PFRs has increased resulting in house dust levels which exceed the levels of polybrominated diphenyl ethers in dust [3,4]. In Belgian dust, the dominating PFRs were tris(2-butoxyethyl) phosphate (TBOEP, 2.03 μg/g), tris(1-chloro-2-propyl) phosphate (TCIPP, 1.38 μg/g), and triphenyl phosphate (TPHP, 0.5 μg/g) [4]. As daily ingestion estimates are 20 mg of indoor dust on average, adults could be exposed to amounts as high as 260 ng PFRs per day [4]. Experiments in laboratory animals indicated that PFRs, such as tris(1,3-dichloro-2-propyl) phosphate, tris(2-chloroethyl) phosphate, and tri-*n*-butyl phosphate, undergo hydrolysis after uptake in the body to form dialkyl or diaryl phosphates (DAPs) [5,6]. Indeed, recent publications demonstrated the presence of several DAPs in human urine, including dibutyl phosphate (DBP), bis(2-chloroethyl) phosphate (BCEP), bis(1-chloro-2-propyl) phosphate (BCIPP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), diphenyl phosphate (DPHP), and bis(2-butoxyethyl) phosphate (BBOEP). However, not all these metabolites were combined in a single analytical protocol using urine [7–10].

In this study, we have developed a method based on solid-phase extraction (SPE) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the determination of BCEP, BCIPP, DBP, DPHP, BDCIPP, and BBOEP in human urine. Through the use of an important number of synthesised labelled internal standards, we could properly assess the method performance and the matrix effects for BDICPP and BBOEP. The final optimised method was compared to the GC–MS/MS method proposed by Schindler et al. [7]. Advantages and disadvantages of both methods were evaluated.

* Corresponding author. Tel.: +32 3 265 2498; fax: +32 3 265 2722.
E-mail address: adrian.covaci@ua.ac.be (A. Covaci).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.06.042

Table 1
Fragmentor voltage, collision energies and multi-reaction monitoring (MRM) transitions for the quantifier and qualifier ions of each DAP and the corresponding internal standard in (−)ESI-MS/MS.

Compound	Fragmentor voltage (V)	Collision energy 1 (eV)	Quantifier MRM	Collision energy 2 (eV)	Qualifier MRM
BCEP-d8	60	5	229.0 > 35.0	5	231.0 > 35.0
BCEP	60	5	221.0 > 35.0	5	223.0 > 35.0
BCIPP	60	6	249.0 > 35.0	6	251.0 > 35.0
DPHP-d10	130	27	259.2 > 98.1	17	259.2 > 159.1
DBP	120	22	209.1 > 79.2	9	209.2 > 153.0
DPHP	130	27	249.1 > 93.2	17	249.1 > 155.1
BDCIPP-d10	80	5	329.0 > 35.0	5	327.0 > 35.0
BDCIPP	80	5	318.9 > 35.0	5	316.9 > 35.0
BBOEP-d4	115	27	301.2 > 78.8	14	301.2 > 198.9
BBOEP	115	27	297.1 > 79.0	14	297.1 > 197.3

Finally, we have applied the new LC–MS/MS method to the determination of DAPs in urine samples from a group of volunteers. The purpose of the analysis was to estimate DAP urinary levels in the Belgian population, and to explore if any individual related differences (age, weight, body mass index, gender, smoking, and alcohol consumption) could influence DAP concentrations in urine. This would be a basis for future and more complete biomonitoring studies investigating these factors or keeping them into account.

2. Materials and methods

2.1. Materials

An overview of target compounds and internal standards (IS) is given in Table 1. DPHP, DBP, pentafluorobenzyl bromide and trimethylsilyl diazomethane (TMSDM) were purchased from Sigma–Aldrich (Bornem, Belgium). BBOEP, BCEP, BCIPP, BDCIPP, BBOEP-d4, BCEP-d8, BDCIPP-d10 and DPHP-d10 were synthesised in the Max Planck Institute (Göttingen, Germany). Purity of the standards was checked with ^{1}H NMR, and was more than 97.5% for BCEP, BDCIPP, BBOEP, BCEP-d8, BDCIPP-d10. Purity of BCIPP and DPP-d10 was 91% and 85%, respectively.

Isolute ENV+ (100 mg/3 mL) was purchased from Biotage, while Bond Elut FL (500 mg/3 mL), Bond Elut PSA (500 mg/3 mL), Bond Elut NH2 (50 and 100 mg, 1 mL) were purchased from Agilent Technologies. Strata-X-AW (100 mg/3 mL) was purchased from Phenomenex Inc. (Torrance, CA, USA), while Oasis WAX (60 mg/3 mL) was purchased from Waters (Millford, MA, USA). Ammonium hydroxide (NH_4OH, 29%), ammonium acetate, toluene, acetonitrile (ACN, analytical grade), and methanol (MeOH, analytical grade) were purchased from Merck Chemicals (Darmstadt, Germany). The LC column Synergi Polar-RP (100 mm × 2.0 mm × 2.1 μm) was purchased from Phenomenex Inc. Ultrapure water (18.2 MΩ) was obtained from an Elga LabWater water purification instrument (Saint Maurice, France).

2.2. Samples

This study analysed a subset of samples from the project Endorup which investigated the presence of various environmental contaminants and their metabolites in human urine. The global study was approved by the local Ethical Committee (Belgian Registry number B30020097009) and registered at clinicaltrials.gov (number NCT01778868) [11]. Morning spot urine samples were taken from a group of adult volunteers (n = 59) and collected in glass tubes. The group consisted of 23 men, 36 women, with a mean age of 40.8 years, a mean body mass index (BMI) of 34.5. Approximately 20% of the volunteers were smokers, 15% ex-smokers and alcohol consumption was also registered. The samples were stored at −20 °C until further analysis. The pooled urine sample for validation purposes was made by combining 5 mL of urine from 25 samples from this set.

2.3. Methods

2.3.1. Sample preparation

2.3.1.1. Optimisation. One mL urine obtained from each single person was added to an empty tube, adjusted to pH 5 with sodium acetate buffer (pH 4.5) and spiked with 120 ng DAPs. The sample was transferred to the SPE cartridges (Oasis WAX, Bond Elut NH_2 50 and 100 mg), the cartridge was washed with 1 mL water (fraction 1), 1 mL 50% MeOH in water (fraction 2), and DAPs were eluted with two times 2 mL 5% NH_4OH in MeOH (fractions 3 and 4). Tests were run in duplicate, all fractions were collected separately and 20 ng IS was added to each fraction before LC–MS/MS analysis. Next, Oasis WAX was tested for (matrix) capacity, washing and eluting conditions. Standard conditions were: 1 mL of urine (adjusted to pH 5 and spiked with 120 ng DAPs), washing with 30% MeOH in water adjusted to pH 5, eluting with 5% NH_4OH in MeOH. One of the steps was varied at the time (in duplicate): loading with 2, 3, or 4 mL urine; washing with 2 mL of 10% or 50% or 1 mL of 80% or 100% MeOH; eluting with 2.5% or 10% NH_4OH in MeOH. For comparison, the capacity test was also run by loading 1 or 4 mL of blank urine and adding 120 ng DAPs to the extract. Strata-X-AW cartridges were also compared by loading 1 mL of spiked urine, washing with 2 mL 30% MeOH (pH 5, to keep the sorbent positively charged) and eluting with 3 mL 5% triethylamine in MeOH, since a stronger base is required for this sorbent [8].

2.3.1.2. Derivatisation. In an attempt to increase the retention and sensitivity of the DAPs on the LC/MS/MS system, SPE extracts were methylated using TMSDM (Fig. 1) before analysis. For this purpose, the extracts were evaporated until dryness, resolubilised in 50 μL of MeOH and 0.2 M TMSDM in ACN, sealed and placed in an oven at 100 °C for 120 min. After the incubation, each extract was carefully evaporated until dryness using a gentle N_2 stream and reconstituted in 150 μL of 50% MeOH in water. Instrumental analysis was done using LC–ESI(+)MS/MS.

2.3.1.3. Final procedure. A volume of 2 mL urine from the individually collected urine samples was spiked with 15 ng of IS and adjusted to pH 5 using a 0.1 M sodium acetate buffer. Oasis WAX cartridges were pre-washed using 2 mL of 5% NH_4OH in MeOH followed by 3 mL of sodium acetate buffer pH 4.5. The urine was quantitatively transferred to the cartridges using 1.0 mL of diluted sodium acetate buffer (pH 4.5). Interferences were removed using 30% MeOH at pH 5. DAPs were eluted with 2 mL of 5% NH_4OH in MeOH. The MeOH extract was collected and evaporated to dryness. Extracts that would not be derivatised were reconstituted in 150 μl of 15% MeOH in water.