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Environmental exposure to pharmaceuticals: A new technique for trace analysis of carbamazepine and its metabolites in human urine☆

Ganna Fedorova [a, b, c, 1], Julius Ben Ari [d, 1], Galit Tadmor [a, b], Ora Paltiel [b, e], Benny Chefetz [a, b, *]

[a] Department of Soil and Water Sciences, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, 76100, Israel
[b] The Hebrew University Center of Excellence in Agriculture and Environmental Health, P.O. Box 12, Rehovot, 76100, Israel
[c] Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zatisi 728/II, 389 25, Vodnany, Czech Republic
[d] The Interdepartmental Equipment Facility, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, 76100, Israel
[e] Braun School of Public Health and Community Medicine of the Faculty of Medicine, Hadassah/Hebrew University Medical Center, Jerusalem, Israel

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ABSTRACT

Pharmaceutically active compounds are taken up and accumulate in crops irrigated with treated wastewater. This raises the concern of chronic human exposure to pharmaceuticals via food consumption. Thus, there is a need to develop a reliable technique to detect and quantify pharmaceuticals at environmentally relevant concentrations in human biological matrices, particularly urine. In this study, we focus on carbamazepine, an antiepileptic drug and recalcitrant compound that is taken up by crops—making it an excellent model compound for this study. This paper presents a new analytical technique enabling quantification of trace concentrations of carbamazepine and its metabolites in the urine of individuals who have been environmentally exposed. Sample preparation included extraction with acetonitrile followed by clean-up through mixed-mode ion-exchange cartridges and analysis using LC/MS/MS. This technique, which was validated for a wide range of concentrations (5–2000 ng L^{-1}), exhibits low limits of quantification (3.0–7.2 ng L^{-1}), acceptable recovery levels (70–120%), and low relative standard deviation (<20%). Unlike currently available methods for the analysis of water or treated wastewater that require large volumes (up to 1 L), the new method uses only 10 mL of urine. Moreover, relative to available methods for carbamazepine detection in the urine of individuals who are chronically treated with this drug, the limit of quantification values with our method are six orders of magnitude lower. The newly developed method has been successfully applied for the quantification of carbamazepine and its metabolites in the urine of healthy people exposed to this pharmaceutical through their diet. Our analytical protocol can provide the scientific community and stakeholders with real data for risk assessments and the design of policies ensuring safe use of wastewater for crop irrigation.

1. Introduction

To meet the mounting water demands by municipal and industrial sectors, treated wastewater is used as an alternative water source in the agricultural sector. In arid and semi-arid zones such as the Middle East, parts of India and China, Mexico and southern regions of the United States, treated wastewater is used as a valuable source for crop irrigation (Sato et al., 2013). However, even when treated wastewater meets irrigation standards, it contains a wide array of organic pollutants, including active pharmaceutical compounds and personal care products, hormones, endocrine-disrupting chemicals, plasticizers, surfactants, fire retardants, perfluorinated compounds, synthetic musks and pesticides (Al-Odaini et al., 2010; Cahill et al., 2011; Ghosh et al., 2010; Kasprzyk-Hordern et al., 2009; Lee et al., 2010; Pan et al., 2011; Robert-Peillard et al., 2015; Woudneh et al., 2015). The presence of these organic pollutants in the irrigation water raises safety concerns regarding

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* Corresponding author. The Hebrew University of Jerusalem, Israel.
E-mail address: benny.chefetz@mail.huji.ac.il (B. Chefetz).
[1] Authors contributed equally.

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possible exposure of the population to them through consumption of wastewater-irrigated crops.

Pollutants of emerging concern, such as pharmaceutical compounds and personal care products, have been the focus of many studies (Nakada et al., 2007; Oliveira et al., 2015; Pennington et al., 2015; Qin et al., 2015). These compounds are not effectively removed by wastewater treatment; thus they are ubiquitous in effluents (Bartelt-Hunt et al., 2009; Jones-Lepp et al., 2012; Yargeau et al., 2014; Zhang et al., 2008), as well as in the receiving environment (Duan et al., 2013). The antiepileptic drug carbamazepine (CBZ) is one of the most frequently detected pharmaceuticals in aquatic environments, including effluent wastewater (Bahlmann et al., 2014), surface water (Zhang et al., 2007), groundwater (Ternes et al., 2007) and even drinking water (Guo and Krasner, 2009). In addition to the parent compound, metabolites of CBZ are also found in wastewater effluents (Bahlmann et al., 2014). Negative effects of CBZ on certain aquatic species have already been shown (Ferrari et al., 2003; Li et al., 2010) and in recent years, a growing number of reports have suggested that CBZ is taken up by crops irrigated with treated wastewater (Goldstein et al., 2014; Malchi et al., 2014; Wu et al., 2013). CBZ is known to be highly stable in the environment (Grossberger et al., 2014; Tixier et al., 2003) and thus can be a suitable marker for anthropogenic pollution, as well as for human exposure to pharmaceuticals via consumption of crops irrigated with treated wastewater.

To assess human exposure to CBZ and other xenobiotics via dietary consumption of fruits and vegetables, it is important to develop a reliable technique for the detection and quantification of trace concentrations of these compounds in urine samples. Several analytical methods for the quantification of different pharmaceuticals in environmental samples have been recently developed (Chen et al., 2015; Fick et al., 2010; Huerta et al., 2013; Miao et al., 2005; Tanoue et al., 2014). Environmentally relevant limits of quantification (low ng g^{-1} and ng L^{-1} ranges) have been reported. However, those methods have been applied for analysis of CBZ in water, biosolids, fish muscle, liver and bile; none have been—nor can they be—used to analyze urine. Moreover, most of the available methods for analysis of pharmaceutical compounds, including CBZ, in water require large sample volumes (100–1000 mL) to ensure a sufficient level of the analyte. This cannot be applied to urine which is obtained in limited volumes (typically <100 mL). On the other hand, available methods for analysis of CBZ in the urine (Rani et al., 2012; Rezaee and Mashayekhi, 2012) of patients who are being chronically treated with this drug (daily doses of 400 to 1000 mg) are not applicable because they deal with concentrations that are six orders of magnitude higher than what is expected for environmental exposure. Therefore, the objective of this study was to develop a reliable technique for the detection and quantification of CBZ and its metabolites in human urine at trace (i.e., nanogram per liter) levels to quantify environmental exposure of this drug.

2. Methodology

Technique development included the following steps: 1) sample extraction and clean-up procedure; 2) analysis of CBZ and its metabolites by LC/MS/MS; 3) method validation, and 4) applicability of the developed technique to real samples.

2.1. Materials and analytical standards

Analytical standards of 10,11-epoxy-10,11-dihydro-carbamazepine (EP-CBZ; 96% purity), 2-hydroxy-carbamazepine (2-OH-CBZ; 98%), 3-hydroxy-carbamazepine (3-OH-CBZ; 98%), 10,11-dihydro-10-hydroxy-carbamazepine (10-OH-CBZ; 98%), 10,11-dihydro-trans-10,11-dihydroxy-carbamazepine (DiOH-CBZ; 97%), 10,11-dihydro-10-hydroxy-CBZ glucuronide (CBZ-O-glucuronide, 98%), 10-OH-CBZ-D3 (99.4%), EP-CBZ-D8 (98%) and CBZ-D2-^{13}C (99%) were obtained from Toronto Research Chemicals Inc. (North York, Canada). CBZ (97% purity) was purchased from Sigma–Aldrich Israel Ltd. (Rehovot, Israel). Weak anion exchange and weak cation exchange Strata X anion weak (AW) and cation weak (CW), respectively (33 μm, 200 mg) and Strata X polymeric reversed-phase sorbent (33 μm, 500 mg) were purchased from Phenomenex Inc. (Torrance, CA). β-Glucuronidase (*Escherichia coli* type IX-A, 125 kU) for deconjugation of CBZ glucuronide was purchased from Sigma–Aldrich Israel Ltd.

2.2. Sample extraction and clean-up procedure

For the sample extraction and clean-up procedure, blank samples (deionized water and urine free of CBZ and its metabolites) and urine samples fortified to 10 and 200 ng L^{-1} (native compound and labeled compound, respectively) were used. Urine samples (10 mL) were frozen in liquid nitrogen and then freeze–dried. Acetonitrile (3 mL) was added to the freeze–dried urine samples followed by 5 min sonication. The liquid phase (extract) was transferred to a 10 mL glass tube containing 1 mL of 20 mM ammonium acetate buffer (pH 6–7). Further clean-up of extracts was carried out using Strata X CW and Strata X AW cartridges. Before use, the cartridges were washed with 2 mL of MeOH and pre-conditioned with 2 mL of acetonitrile/buffer (75/25 v/v). The extracts were loaded onto the solid-phase extraction (SPE) cartridges: Strata X CW was connected with an SPE tube adapter to Strata X AW. The eluents were evaporated to dryness and then re-dissolved in 100 μL of mobile phase (water/acetonitrile, 80/20) acidified with 1% acetic acid and analyzed by LC/MS/MS.

We also quantified the level of CBZ-O-glucuronide in urine samples as it is one of the most abundant metabolites of CBZ excreted in the urine. A de-conjugation test was conducted by incubating urine samples with β-glucuronidase (5 kU of enzyme per 10 mL sample) at 37°C overnight. Thereafter the samples were treated as described above.

2.3. LC/MS/MS analysis

Analysis of CBZ and its metabolites was carried out using an Agilent 1200 Rapid Resolution LC system coupled to an Agilent 6410 triple quadruple mass spectrometer (Agilent Technologies Inc., Santa Clara, CA). Target analytes were separated on an Acclaim C18 RSLC column (2.1 × 150 mm, particle size 2.2 μm, Dionex). A gradient of acetonitrile and water (acidified with 0.1% acetic acid) was used for the separation of target compounds (see Table S1, Supplementary material). All analytes were ionized using an electrospray interface in positive ion mode. The following parameters were used for the mass spectrometer: capillary voltage 4000 V; drying gas (nitrogen) temperature and flow 350 °C and 10 L min^{-1}, respectively; nebulizer pressure 35 psi; nitrogen (99.999%) was used as a collision gas. The m/z ratios for precursor and product ions of target analytes, as well as collision energies and retention times, are presented in Table S2, Supplementary material.

2.4. Validation of the developed technique

The method was validated by considering its linearity, limit of quantification (LOQ), recovery and precision. Calibration curves for all analytes in the range 0.05–100 μg L^{-1} were prepared using water/acetonitrile (80/20) acidified with 1% acetic acid. The lowest standard concentration in the calibration curve was considered the LOQ. The LOQ response should be 10 times higher than that of the average noise in the chromatogram, and identifiable and