Occurrence of selected pharmaceuticals in flooded arable soil: Bioaccumulation in root vegetables and health risk assessment

Škrbić B.,[1*] Živančev J.,[1] Antić I.,[1] Buljovčić M.[1] And Bayona J. M.2

[1]University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Republic of Serbia

[2]Institute of Environmental Assessment and Water ResearchJordi Girona, 1808034 Barcelona, Spain

*corresponding author: Prof. Dr. Biljana Škrbić

e-mail: biljana@tf.uns.ac.rs

Abstract: The presence of 12 pharmaceuticals (analgesics/anti-inflammatories: *ibuprofen, salicylic acid, diclofenac*; lipid regulator and cholesterol lowering statin drug: *benzafibrate*; psychiatric drug: *carbamazepine*; histamine H2 receptor antagonists: *ranitidine*; β-blocking agent: *propranolol*; diuretic: *hydrochlorothiazide*; antihypertensive: *losartan*; antibiotics: *erythromycin, clarithromycin*; calcium channel blocker: *diltiazem*) were determined in flooded arable soil samples collected from region in the northern Serbian province of Vojvodina heavily flooded during May 2014, when exceptionally heavy rains fell on Serbia and many cities and villages were completely under water. This region was selected for the investigation as it is the area of intensive agricultural production. Additionally, samples of potato and carrot were collected and analysed on the same pharmaceuticals, as uptake of selected pharmaceuticals by root vegetables may represent a worst case scenario of direct contact between the flooded soil and the consumed crops. Samples were prepared using solid-phase extraction and the presence of 12 pharmaceutical compounds in the extracts was analyzed by ultra-high performance liquid chromatography coupled to triple quadruple mass spectrometry (UPLC—MS/MS). Taking into account that contents of selected pharmaceuticals were below limit of detection, the health risk associated with the target compounds in analysed vegetables should not be of concern for the Serbian consumers.

Keywords: Pharmaceuticals, flooded arable soil, bioaccumulation, health risk

1. Introduction

Pharmaceuticals cover a large group of substances that belong to different chemical families. These compounds are introduced into the environment as a result of medical and veterinary use. Among the most frequently occurring drug classes in the environment are antibiotics, nonsteroidal anti-inflammatories, and anti-convulsants, with concentrations between ng/L to low μg/L in treated wastewater and μg/kg to low mg/kg (dry weight) in biosolids. Recognized pathways for the entrance of pharmaceuticals to the environment and subsequently to the human food web are application of manure (veterinary pharmaceuticals) (Campagnolol *et al*., 2002; Zhao *et al*., 2010) or sewage sludge (Onesios *et al*., 2009; McClellan and Halden, 2010; Wu *et al*., 2010) (human pharmaceuticals) as soil fertilizer or amendments or via irrigation of fields with wastewater containing pharmaceutical residues (Pedersen *et al*., 2005; Wu *et al*., 2010). Hence, the presence of pharmaceutical compounds and their metabolites in the environment has raised concern due to the potential ecological and health risks associated with exposure to these pollutants (Cunningham *et al*., 2010; Kostich *et al*., 2014). Although the human risk from dietary intake is expected to be small for individual pharmaceutical compounds, given that numerous pharmaceuticals are present in the environment as a mixture, and that there may be hyposensitive populations, more researches are clearly needed to better understand the occurrence and risk of pharmaceuticals in plants. Moreover, the perceived human exposure is likely to be the greatest for raw-consumed vegetables.

The uptake and bioaccumulation of pharmaceuticals in the edible parts of food crops and fodders and their subsequent entry into the human food chain have been gaining prominence over the last decade. Moreover, numerous studies, mainly conducted under hydroponic or greenhouse conditions, highlighted pharmaceuticals uptake and bioaccumulation in plants exposed to known concentrations of individual or cocktails of pharmaceuticals (Herklotz *et al*., 2010; Wu *et al*., 2010; Karnjanapiboonwong *et al*., 2011; Shenker *et al*., 2011; Calderon-Preciado *et al*., 2012; Eggen and Lillo, 2012; Sabourin *et al*., 2012; Tanoue *et al*., 2012; Wu *et al*., 2013; Christou *et al*., 2016, 2017).

In this context, the aim of this study was to determine the presence of 12 pharmaceuticals (belonging to the different therapeutic groups) in flooded arable soil and their uptake and bioaccumulation in root vegetables (potato and carrot), as well as the estimation of the potential health risk of general population through the daily consumption of the studied plant.

2. Materials and methods

All pharmaceutical standards were of high purity grade (>90%). The solvent, HPLC grade methanol and formic acid 98% were provided by Merck (Darmstadt, Germany).

Nitrogen for drying 99.9990% of purity was from Messer Tehnogas A.D. (Belgrade, Serbia). A Mili-Q-Advantage system from Millipore, Molsheim (France) was used to obtain HPLC-grade water. The cartridges used for solid phase extraction (SPE) were Oasis HLB (200 mg, 6 mL) from Waters Corporation (Milford, MA, U.S.A.).

2.1 Sampling and sample preparation

Sampling was performed in autumn 2016. A total of 21 topsoil samples (0-30 cm) were collected from selected locations. Each sample was a composite of 10 subsamples collected from a 100 m x 100 m grid using a stainless steel hand trowel and transported to the laboratory. Subsamples were thoroughly mixed to provide a composite sample of 3 kg of soil. Soil samples were air-dried at room temperature (25 ^{O}C), then passed through a 2 mm polyethylene sieve and finally ground into fine powder with a pestle. The ground samples were stored (at 4 ^{O}C) in hermetically sealed polyethylene bags for further analysis. Potato and carrot samples were collected randomly from each selected locations. Samples of potato and carrot were then thoroughly washed with tap water followed by rinsing with ultrapure deionized water to remove any soil particles, blotted dried with tissue paper and stored in plastic bags at 18 ^{O}C until analysis.

The method previously developed by Jelić et al. (2009) was slightly modified and applied for pharmaceuticals determination in soil. The soil samples were extracted by pressurized liquid extraction (PLE) using ASE 350 accelerated solvent extractor (Dionex, Sunnyvale, CA) equipped with 33 ml stainless extraction cells. About 5 g of previously homogenized samples was weight in the extraction cells. The extraction method was established with the following parameters: methanol/water, 1/2 (v/v) as extraction solvent, temperature of 90 ^{O}C, a preheating period of 5 min, 3 static cycles, each lasting 7 min, total flush volume of 100% of cell with 80 s of nitrogen purge. The extract obtained in PLE was ~80 ml. Aliquot of 20 ml was diluted with 600 ml of HPLC water (methanol < 5%), and processed by SPE. Oasis HLB cartridges (200 mg, 6 ml) were used for clean-up. The cartridges were conditioned with 5 ml of methanol followed by 5 ml of HPLC water at neutral pH. Then the dilution of ASE extract was percolated through the cartridges using a Baker vacuum system (J.T. Baker, The Netherlands). Finally, the compounds were eluted with 8 ml of methanol at a 1 ml/min flow and then the SPE extracts were evaporated under a nitrogen stream and reconstituted with 1 ml of methanol–water mixture (25:75, v/v). Prior to the LC–MS/MS analysis, the samples were passed through 0.22 μm filters.

The method applied for preparation vegetable crops is previously used by (Wu et al. 2012).

2.2. Instrumental analysis

Instrumental analysis of all samples was done by high performance liquid chromatography coupled to triple quadrupole mass spectrometry (UHPLC–MS/MS). Hypersil GOLD™, 50 × 2.1 mm i.d., 1.9 μm column (Thermo Fisher Scientific, USA) was used with a flow rate of 0.4 ml/min, and the column temperature was maintained at 30°C. The injection volume was 10 μL. The mobile phase consisted of eluent A containing water/formic acid (99.9:0.1, v/v), and eluent B consisting of methanol/formic acid (99.9:0.1, v/v). The gradient program started with 5% of eluent B, increasing to 95% in 4.5 min, raising to 100% in the following 0.1 min and held 1.9 min and then back to initial conditions (7.0 min; holding time 1.0 min). Total time of the run was 8 min. For analytes detection, triple quadrupole mass spectrometer (MS/MS) TSQ Vantage (Thermo Fisher Scientific, USA) equipped with heated-electrospray ionization probe (HESI-II, Thermo Scientific, USA) was used. Parameters of the ion source were as follows: spray voltage - 3.4 kV, vaporizer temperature - 250°C, sheat gas presssure - 40 arbitrary units, auxiliary gas pressure- 10 arbitrary units, and capillary temperature - 270°C.

2.3 Quality control

Calibration curves were generated using linear regression analysis and showed good fits (r^2>0.9900) over the established concentration points ranging from 1, 5, 10, 20, to 50 or 100 μg/L depending of the compound. Quantification of target analytes, based on peak area, was performed by the matrix-matched approach. Limit of detection (LOD) and limit of quantification (LOQ) were determined for analysed samples as the minimum detectable amount of analyte with a signal-to-noise ratio of 3 and 10, respectively. Validation of the analytical method was carried out by determination of recoveries (″in-house″) of uncontaminated sample spiked at level of 20 μg/kg for soil and 5 μg/kg for vegetable crops (for each of compounds). Precision of the method was determined by calculating the relative standard deviation (%RSD) of the triplicate spiked samples.

3. Results and discussion

UHPLC-MS/MS parameters of target compounds, recovery and repeatability values of multi-residue method obtained for analyzed samples are presented in Table 1. The recovery results are presented as average values of three replicates. Table 1 demonstrates the applicability of the matrix-matched calibration curves for majority of the pharmaceuticals and the investigated samples because the satisfactory recovery values (between 66% to 118%) were obtained. The method was applied to the simultaneous determination of the studied pharmaceuticals in soil and vegetable samples collected from region in the northern Serbian province of Vojvodina heavily flooded during May 2014. The quantification of real samples was performed against the corresponding matrix-matched calibration curves approved with acceptable R^2. Results obtained in this study showed a weak contamination of analyzed samples i.e. in all investigated samples of soil and vegetables, levels of selected pharmaceuticals were below the determined LODs.

Table 1. UHPLC-MS/MS parameters of target compounds under optimized conditions on Accela - TSQ Vantage (Thermo Fisher Scientific, USA). Parameters indicating the performance of the analytical method: limit of detection (LOD), limit of quantification (LOQ) and recoveries obtained for target compounds in all matrices studied.

Therapeutic group	Compounds	Precursor ion (m/z)	Product ions[a] (m/z)	CID[b] (eV)	LOD[c/d] (ug/kg)	LOQ[c/d] (ug/kg)	Recovery[c/d] (%)	RSD[c/d] (%)
Analgesics/anti-inflammatories	Ibuprofen	205.91 [M-H]⁻	162.09	10	0.41/0.25	1.37/0.83	85/92	3/5
	Salicylic acid	137.97 [M-H]⁻	94.20/66.30	19/33	0.07/0.03	0.25/0.11	71/82	4/8
	Diclofenac	250.96 [M-H]⁻	214.90	19	0.02/0.04	0.06/0.13	67/71	15/11
Lipid regulator and cholesterol lowering statin drug	Benzafibrate	360.48 [M-H]⁻	274.77/154.92	21/30	0.01/0.01	0.04/0.04	96/93	3/9
Psychiatric drug	Carbamazepine	237.81 [M+H]⁺	194.96/193.96	20/33	0.01/0.03	0.3/0.1	77/72	13/8
Histamine H2 receptor antagonists	Ranitidine	315.65 [M+H]⁺	174.46/131.09	17/33	0.03/0.01	0.11/0.03	84/81	4/7
β-blocking agent	Propranolol	260.80 [M+H]⁺	117.20/183.98	19/18	0.01/0.01	0.03/0.03	79/85	4/7
Diuretic	Hydrochlorothiazide	296.51 [M-H]⁻	269.60/205.81	20/24	0.03/0.11	0.11/0.36	118/95	1/5
Antihypertensive	Losartan	421.34 [M-H]⁻	127.34/179.21	10/14	0.12/0.83	0.40/2.76	66/87	5/4
Antibiotics	Erythromycin	734.43 [M+H]⁺	576.45/159.01	17/30	0.004/0.08	0.01/0.27	66/77	2/4
	Clarithromycin	748.45 [M+H]⁺	159.01/576.45	29/17	0.003/0.003	0.01/0.01	78/82	7/11
Calcium channel blocker	Diltiazem	415.39 [M+H]⁺	178.93/110.1	24/59	0.002/0.05	0.01/0.17	71/78	10/6

[a]Numerical values are given in the order quantifier/qualifier ion.

[b] Collision-induced dissociation energy for quantifier/qualifier ion.

[c]Values given for soil samples

[d]Values given for vegetables samples

4. Conclusions

The multi-residue method is developed for simultaneous determination of 12 pharmaceuticals from different therapeutic classes in extract of soil and vegetable crops. The described analytical method allowed the simultaneous extraction of all compounds, demonstrating the applicability and effectiveness of PLE followed by SPE clean-up step. Recoveries of the developed method were satisfactory for most compounds (between 66% to 118%) with RSD values lower than 15%. The satisfactory recoveries were obtained for the most of pharmaceuticals using matrix-matched calibration curves. The applicability of the method was demonstrated on real samples of soil and vegetables collected in a region of northern Serbian province of Vojvodina.

References

Calderon-Preciado, D., Renault, Q., Matamoros, V., Ca nameras, N. and Bayona, J.M. (2012), Uptake of organic emergent contaminants in spath and lettuce: an in vitro experiment, *Journal of Agricultural and Food Chemistry,* **60** (8), 2000-2007.

Campagnolol, E.R., Johnson, K.R., Karpati, A., Rubin, C.S., Kolpin, D.W., Meyer, M.T., Esteban, J.E., Currier, R.W., Smith, K., Thu, K.M. and McGeehin, M. (2002), Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations, *Science of the Total Environment*, **299**, 89–95.

Cunningham, V.L., Perino, C., D'Aco, V.J., Hartmann, A. and Bechter, R. (2010), Human health risk assessment of carbamazepine in surface waters of North America and

Europe, *Regulatory Toxicology and Pharmacology*, **56**, 343-351.

Christou, A., Antoniou, C., Christodoulou, C., Hapeshi, E., Stavrou, I., Michael, C., Fatta-Kassinos, D. and Fotopoulos, V. (2016), Stress-related phenomena and detoxi- fication mechanisms induced by common pharmaceuticals in alfalfa (Medicago sativa L.) plants, *Science of the Total Environment*, **557-558**, 652-664.

Christou, A., Karaolia, P., Hapeshi, E., Michael, C., Fatta-Kassinos, D. (2017), Long-term wastewater irrigation of vegetables in real agricultural systems: Concentration of pharmaceuticals in soil, uptake and bioaccumulation in tomato fruits and human health risk assessment, *Water Research*, **109**, 24-34.

Eggen, T. and Lillo, C. (2012), Antidiabetic II drug metformin in plants: uptake and translocation to edible parts of cereals, oily seeds, beans, tomato, squash, carrots, and potatoes, *Journal of Agricultural and Food Chemistry*, **60** (28), 6929-6935.

Herklotz, P.A., Gurung, P., Vanden Heuvel, B. and Kinney, C.A. (2010), Uptake of human pharmaceuticals by plants grown under hydroponic conditions, *Chemosphere,* **78** (11), 1416-1421.

Jelić, A., Petrović, M. and Barceló, D. (2009), Multi-residue method for trace level determination of pharmaceuticals in solid samples using pressurized liquid extraction followed by liquid chromatography/quadrupole-linear ion trap mass spectrometry, *Talanta*, **80**, 363–371.

McClellan, K. and Halden, R.U. (2010), Pharmaceuticals and personal care products in archived U.S. biosolids from the 2001 EPA national sewage sludge survey, *Water Research*, **44**, 658–668.

Onesios, K.M., Yu, J.T. and Bouwer, E.J. (2009), Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review, *Biodegradation,* **20**, 441–466.

Karnjanapiboonwong, A., Chase, D.A., Canas, J.E., Jackson, W.A., Maul, J.D., Morse, A.N. and Anderson, T.A. (2011), Uptake of 17-ethynylestradiol and triclosan in pinto bean, Phaseolus vulgaris. *Ecotoxicology and Environmental Safety,* **74** (5), 1336-1342.

Kostich, M.S., Batt, A.L. and Lazorchak, J.M. (2014), Concentrations of prioritized pharmaceuticals in effluents from 50 large wastewater treatment plants in the US and implications for risk estimation, *Environmental Pollution*, **184**, 354-359

Zhao, L., Dong, Y.H. and Wang, H. (2010), Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China, *Science of the Total Environment*, **408**, 1069–1075.

Pedersen, J.A., Soliman, M. and Suffet, I.H. (2005), Human pharmaceuticals, hormones, and personal care product ingredients in runoff from agricultural fields irrigated with treated wastewater, *Journal of Agricultural and Food Chemistry,* **53**, 1625–1632.

Sabourin, L., Duenk, P., Bonte-Gelok, S., Payne, M., Lapen, D.R. and Topp, E. (2012), Uptake of pharmaceuticals, hormones and parabens into vegetables grown in soil fertilized with municipal biosolids. *Science of the Total Environment*, **431** (0), 233-236.

Shenker, M., Harush, D., Ben-Ari, J. and Chefetz, B. (2011), Uptake of carbamazepine by cucumber plants - a case study related to irrigation with reclaimed wastewater, *Chemosphere,* **82** (6), 905-910.

Tanoue, R., Sato, Y., Motoyama, M., Nakagawa, S., Shinohara, R. and Nomiyama, K. (2012), Plant uptake of pharmaceutical chemicals detected in recycled organic manure and reclaimed wastewater. *Journal of Agricultural and Food Chemistry,* **60** (41), 10203-10211.

Wu, C., Spongberg, A.L., Witter, J.D., Fang, M. and Czajkowski, K.P. (2010), Uptake of pharmaceutical and personal care products by soybean plants from soils applied with biosolids and irrigated with contaminated water, *Environmental Science & Technology,* **44**, 6157–6161.

Wu, C., Spongberg, A.L., Witter, J.D., Sridhar, B.B.M., (2012), Transfer of wastewater associated pharmaceuticals and personal care products to crop plants from biosolids treated soil, *Ecotoxicology and Environmental Safety*, **85**, 104–109.

Wu, X., Ernst, F., Conkle, J.L. and Gan, J. (2013), Comparative uptake and translocation of pharmaceutical and personal care products (PPCPs) by common vegetables, *Environment International*, **60**, 15-22.