# ASSESSING THE FATE OF PHARMACEUTICAL RESIDUES IN CROPS IRRIGATED WITH WASTEWATER USING A MULTI-RESIDUE METHOD BASED ON LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

**HAPESHI E.[1], CHRISTOU A.[2], MARATHEFTIS G.[2], MICHAEL C.[1] and FATTA-KASSINOS D.[1]**

[1] Nireas-International Water Research Center and Department of Civil and Environmental Engineering, School of Engineering, University of Cyprus, Nicosia, Cyprus,
[2] Agricultural Research Institute, Nicosia, Cyprus
E-mail: dfatta@ucy.ac.cy, hapeshi.evroula@ucy.ac.cy

## ABSTRACT

Many arid and semi-arid regions around the Mediterranean basin like Cyprus, historically suffer from water scarcity, a fact that results in the need to exploit new sources of water. The need for increasing the acceptance of reuse in agriculture and for groundwater recharge is apparent. However, during the last years, there is a great concern among scientists concerning the transfer and accumulation of contaminants of emerging concern into the food chain through crop uptake which poses a potential risk to human health. The objective of this work was to evaluate the existence of three pharmaceutical residues in tomatoes' crops irrigated with reclaimed water and tubewell water in a greenhouse experiment. A rapid, sensitive multi-residue analytical method has been developed and optimized for the simultaneous quantification of diclofenac (DCF), trimethoprim (TMP) and sulfamethoxazole (SMX). The analytical method consisted of preconcentration and clean-up by solid phase extraction (SPE) followed by ultra-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC–MS/MS) operated in multiple-reaction monitoring mode. Average recoveries of the three analytes from fortified samples ranged between 70.1% and 110.0%, with relative standard deviations (RSD) lower than 9.4%. The concentration of the contaminants in the crops ranged from 15 to 150 μg $Kg^{-1}$ (dry weight). The highest concentration level in crops has been found for DCF which is one of the non-antiinflamatory drugs consumed the most in Cyprus. The proposed method is fast, sensitive and easy to perform, making it applicable for high-throughput monitoring of pharmaceutical residues in tomatoes.

**Keywords**: Solid phase extraction, contaminants of emerging concern, reclaimed water, tomatoes, liquid chromatography tandem mass spectrometry, uptake

## 1. Introduction

As water scarcity is aggravated by climate change, the reclaimed wastewater is considered as an attractive alternative source of fresh water for agricultural irrigation (Wu *et al*., 2010; Wu *et al*., 2013; Kinney *et al*., 2006; Navon *et al*., 2011; Calderón-Preciado *et al*.). Reuse has already been used in many countries, such as the USA, Israel, Spain, Mexico, Australia, Singapore and Cyprus (Calderon-Preciado *et al*., 2013; Wu *et al*., 2013; Chefetz *et al*., 2008;Herklotz *et al*. 2010; Greenway and Simpson, 1996). The utilization of treated urban wastewater either for irrigation purposes or groundwater replenishment due to prolonged drought periods constitutes a very important activity on a national level in Cyprus. Currently, there is an increasingly growing momentum towards the reuse of wastewater while at the same time the concern with respect to the existence of organic micropollutants including pharmaceutical residues in the treated wastewater effluents follows also an increasing trend.

Over the last decades, several studies show that many xenobiotic compounds, including pharmaceutical residues and personal care products (PPCPs), are commonly present in the treated effluents and in the biosolids of the wastewater treatment plants (UWTPs) (Gros *et al*., 2006; Fatta-Kassinos *et al*., 2011; E. Hapeshi *et al*., 2014; Ternes, 1998; Vanderford and Snyder, 2006; Miao *et al*., 2005). Land application of these biosolids and the reuse of treated wastewater, led to the indroduction of such contaminants into the terrestrial and aquatic environments (Wu *et al*., 2010). Therefore, the fate of various organic compounds and especially pharmaceutical residues in treated wastewater and their possible transfer into food products causes an unknown human health risk.

As Christou *et al*. (2014) reported, 22 million cubic meters of treated effluent is produced in Cyprus, from which almost 65% is used for the irrigation for several plants such as citrus, olives and vegetables, following the national code of practice for reuse of reclaimed wastewater. The objective of this study was to develop a validated analytical methodology to investigate the uptake and translocation of three pharmaceutical residues, most commonly occurring in wastewater by the irrigated crop of tomato with treated wastewater from two Urban wastewater treatment plants (UWTP I and UWTP II) in Cyprus, which apply activated sludge and membrane bioreactor treatment, respectively. The general goal of this study was to assess the chemical quality of tubewell water and secondary-treated effluent used for agricultural irrigation and to evaluate effect on the uptake of contaminants of emerging concern in irrigated tomatoes which were grown under field conditions. Three contaminants, i.e. diclofenac, trimethoprim and sulfamethoxazole were selected for the development/optimization of the analytical method. The selection of the compounds was based on the levels of their residual concentration of pharmaceutical residues in the specific wastewater flows.

## 2. Materials and methods

### 2.1. Sampling

Sandy clay loam soil located at the experimental station of the Agricultural Research Institute in Nicosia, Cyprus, was used for the growing of the tomatoes plants. For their irrigation of tomatoes' plants, three different water sources were used; tertiary treated effluent of UWTP I and UWTP II, as well as tube well water (TW) which is water abstracted from a nearby borehole within the experimental station. One of the aims of this study was to assess the impact of the reuse of treated effluents produced from two different UWTPs with different treatment process for the irrigation of tomato crops. The first treatment system (UWTP I) includes slow sand filtration and chlorination, while the second one (UWTP II) Membrane Bioreactor (MBR). The duration of the growing period for each year (2011-2014) was 4 months (120 days). A completely randomised block design was applied, while each treatment was independently run in five replicates. The irrigation of crops was performed with drips, based upon direct measurements of soil moisture status (15 centibars) by the use of tensiometers. Almost 105 tones of wastewater from each wastewater treatment plant were used to irrigate 100 tomato plants. This means that over the growing season tomato plants were irrigated with 1 tone of wastewater per plant.

### 2.2. Extraction and LC-MS/MS analysis

Plant samples were extracted with 10 mL of ethanol, assisted by ultrasonic probe (3000 rpm). More specifically, for the extraction of TMP and DCF the pH of ten grams sample S1 was adjusted to 6-7 with the addition of small amount of solid $NaHCO_3$ while for the extraction of SMX the pH of ten grams sample S2 was left as it is (pH 3-4). Ten mL of EtOH was added to each sample and followed by sonication bath for 1h, and centrifugation at 3000 rpm for 30 min. The supernatant was carefully withdrawn and kept, while the residue was resuspended in 10 mL of 1:1 EtOH:$H_2O$, sonicated for 30 min and centrifuged. The combined extracts were then diluted with $H_2O$ to 400 mL (final %EtOH in the extracted liquid should be less than 5%).

The diluted ethanolic tomato extracts from blanks, samples and spiked blank samples was loaded under gravity onto HLB cartridge (OASIS HBL from Waters), which was preconditioned with 5 mL of methanol followed by 5 mL distilled water. The analytes were then eluted with 8 mL of methanol at 1 mL min-1 flow rate. The eluted methanol was evaporated to dry under a gentle nitrogen stream and the residue reconstituted in 1 mL of methanol-water mixture 25:75 v/v.

Chromatographic analysis was performed on an ACQUITY TQD UPLC-MS/MS system (Waters Corporation). A triple quadruple mass spectrometer coupled with electrospray ionisation (ESI) source was used for sample analysis. Data acquisition was performed with MassLynxTM software. Separation of these pharmaceutical compounds was achieved under gradient conditions. Column BEH Shield RP18 (1.7 μm; 2.1×50 mm; Waters) was used for the chromatographic analysis with mobile phase consisting of water + 0.1% formic acid as eluent A and methanol as eluent B. The elution gradient was 0 min 5% B, 1.5 min 5% B, 2 min 30% B, 3 min 50% B, 5 min 70% B, 6 min 90% B, 7 min 90% B, 7.1 min 5% B, 9 min 5% B. The LC analysis conditions were: $T_{column}$ = 40 °C, flow rate = 0.3 mL $min^{-1}$, run time = 9 min, partial loop with needle overfill. The flow rate was 0.3 mL $min^{-1}$, and the injection volume of samples was 20 μL. Data acquisition was performed with positive electrospray in multiple reaction monitoring mode (MRM), recording the transitions between the precursor ion and the most abundant fragment ions. The most abundant transition product ion was typically used for quantification of the target compound while the second transition product together with the ratio of the intensities of the two transitions, were used for confirmation purposes. The accurate mass and composition for the precursor and the fragment ions were calculated using software MassLynxTM incorporated in the instrument. A tandem quadruple detector (TQD) was used for the detection of target analytes. In order to achieve sufficient sensitivity target for the quantitative analysis, data acquisition was performed with ESI in multiple reaction monitoring mode (MRM), recording the transitions between the precursor ion and the most abundant fragment ions.

## 3. Results

### 3.1. Uptake of pharmaceutical residues by tomatoes' plants

This study demonstrates the suitability of UPLC-MS/MS for quantitative routine pharmaceutical residues analysis in tomatoes. The first step of the study was to develop and optimize the methodology of sample preparation and then the process of solid phase extraction of the determination of pharmaceutical compound in tissue plants.

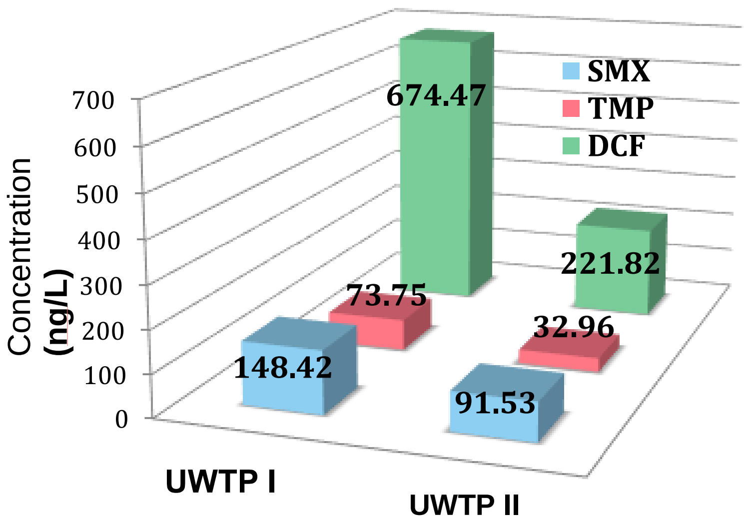


**Figure 1:** Concentration values (ng $L^{-1}$) for target pharmaceuticals in treated wastewater for UWTPI and UWTP II.

For the optimization of the extraction method many experiments were performed to estimate the % recovery of the selected organic compounds from plants. The effect of the pH on the sample preparation was one of the experimental parameters studied to achieve the optimized pH of the extraction method. The crops, which were irrigated with the two different, reclaimed wastewater sources presented the same uptake degree of the pharmaceutical residues by tomatoes' crops. Average recoveries of the three analytes from fortified samples ranged between 70.1% and 110.0%, with relative standard deviations (RSD) lower than 9.4%. More specifically, the average recoveries for TMP, SMX and DCF was 85.9, 70.1 and 110.0, respectively.

Results from this study clearly showed that irrigated crops were capable of taking up the three pharmaceuticals studied, but residues displayed significant disparities in their potential for root uptake and subsequent translocation. DCF was identified and quantified in irrigated crops at levels higher than the other pharmaceutical residues (Figure 1).

Table 1 presents the concentrations of DCF, TMP and SMX in the tomatoes. Results from this study showed that among the three pharmaceuticals considered in this study, DCF accumulated in tomatoes vegetable at levels higher than the other target analytes (DCF=114-150 μg $kg^{-1}$, SMX=21-34 μg $kg^{-1}$ and TMP=13-17 μg $kg^{-1}$). The detailed results of this study will be presented in the full paper.

**Table 1:** DCF, TMP and SMX concentrations (average ± standard deviation) detected in tomatoes' plants grown in sandy soil.

| | **Average concentration μg/Kg** | | |
|---|---|---|---|
| **Samples** | **TMP** | **SMX** | **DCF** |
| **UWTP I** | 13.18±1.32 | 21.52±1.54 | 169.78±3.73 |
| **UWTP II** | 16.96±1.05 | 34.32±1.59 | 114.39±2.31 |
| **TW** | <DL | <DL | <DL |

DL: Detection limit

## 4. Conclusions

This study clearly demonstrates the ability of plants to uptake pharmaceutical residues when exposed to treated effluent, while these organic compounds display disparities in their potential for root uptake and translocation to the other part of the plant. The ability of plants to uptake emerging contaminants depends on several physicochemical properties like the $p_{Ka}$ and the $K_{ow}$ of the contaminants, introducing pathways, interaction with the substrate, etc. The presence of these pharmaceutical residues in crop tissues correlated with the concentrations of pharmaceuticals, which were estimated in treated wastewater. For example, the highest concentrations of pharmaceutical compounds (e.g. DCF) found in secondary-treated wastewater, correlated with their presence in crop tissues.

Results from this study showed that among the three pharmaceuticals considered in this study, diclofenac accumulated in tomato's at levels higher than the other target analyte. According to the available literature, depending of the type of the pharmaceutical, the considered translocation of these emerging contaminants to roots, stems and leaves were varied (Shenker *et al*., 2011). For future work, It would be interesting to gain deeper understanding into the mechanisms of translocation of these contaminants to roots, stems and leaves.

The general conclusion of the current study is that the uptake of active pharmaceutical residues by crops like tomatoes in soils irrigated with reclaimed wastewater is possible and this underlines the potential risks that may be associated with reuse.


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