TRENDS IN USE OF PHARMACEUTICALS, CONTROLLED PSYCHOACTIVE AND ILLICIT DRUGS AS REVEALED FROM THE INFLUENTS’ ANALYSIS OF WWTP OF ATHENS, GREECE

VIOLA L. BOROVA¹, MARILENA E. DASENAKI¹, NIKI C. MARAGOU¹, NIKIFOROS ALYGYZAKIS¹, ATHANASIOS S. STASINAKIS², NIKOLAOS S. THOMAIDIS¹,* and THEMISTOKLIS D. LEKKAS²

¹National and Kapodistrian University of Athens, Department of Chemistry, Panepistimiopolis 15771, Athens, Greece, ²Water and Air Quality Laboratory, Department of Environment, University of the Aegean, University Hill, 81100 Mytilene, Greece
* Corresponding author’s email address: ntho@chem.uoa.gr

ABSTRACT

Pharmaceuticals, psychoactive and illicit drugs and their metabolites are compounds which are considered as environmental emerging contaminants of particular concern. Their residues, after humans’ consumption, can end up in raw wastewater and through sewage system, in the receiving water body. Potential negative effects to wildlife or human can occur, since most of these residues have limited removal and potential pharmacological activities.

The aim of the present study was to develop a method for the determination of more than 150 drugs in wastewater samples (influents) and to evaluate their temporal variations, in order to have instantaneous information on trends in drug use. For achieving those aims, an advanced analytical method for detecting and quantifying more than 150 different drugs in wastewater samples by LC-(ESI)-MS/MS was developed. The method for wastewater analysis involved pre-concentration and clean-up by solid phase extraction (SPE), using two adsorbents (Strata-X and Strata-X-C), followed by LC-MS/MS analysis, with a reversed-phase C18 column (InertSustain) and electrospray ionization (ESI) in both positive and negative modes. The proposed method provided absolute recoveries between 50% (norephedrine, thiopental and SGN) and 113% (Topiramate) and slightly worse, but precise, recoveries, for cannabinoids (10% for cannabidiol to 20% for 11-nor-Δ9-THC acid), metformin and simvastatin (10%), mfenamic acid (18%), atorvastatin (22%), gemfibrozil (35%), ranitidine (41%) and pentobarbital (137%). Recoveries of cannabinoids were corrected with use of labeled internal standards (THCA-d3 and THC-d3). The developed method was applied to 24-h flow proportional composite influent and effluent wastewater samples taken for 8 consecutive days in four different periods/years (December 2010, April 2011, April 2012 and March 2013).

All opioids, NSAIDs, statins, antihypertensive drugs, antipsychotics, antiepileptics, TCAs (Clomipramine), TeCAs, anesthetics (Lidocaine) and barbiturates were determined in all influent samples. Concentrations measured in the influent wastewaters were used to back-calculate drug use at the community level. Removals during wastewater secondary treatment were calculated. Seasonal variation of the selected compounds during the sampling periods in influent samples has shown an increase of methadone, cocaine, cannabis and ecstasy use, while use of antiepileptics was decreased. However, it is notable that during the economic crisis in Greece, between 2010 and 2013, there is a significant increase of use of antidepressants and antipsychotics, like citalopram, lorazepam, clozapine, mirtazapine, and fluoxetine.

Keywords: pharmaceuticals, illicit drugs, metabolites, wastewater, trends, LC-MS/MS
1. INTRODUCTION

Pharmaceuticals, psychoactive and illicit drugs and their metabolites are compounds which are considered as environmental emerging contaminants of particular concern. These substances are present in wastewater (influent and effluent) as a consequence of high volumes of production and use (Castiglioni et al., 2011, Togola et al., 2007). These substances may enter to the sewage system unaltered or as metabolites, after consumption and excretion. Data from the measured concentration of the analyte is often used to back calculate drug usage in local communities (sewage epidemiology), to assess the environmental risk posed to humans and wildlife or to observe usage trends in the communities (Zuccato et al., 2005, Van Nuijs et al., 2011).

Many publications have demonstrated the presence of drugs of abuse and pharmaceuticals as well as their metabolites in the aquatic environment and estimate the usage of these substances, however there are not any detailed information related to the discharge trends in the community level in a long period of investigation.

The aim of the present study was to develop a method for the simultaneous determination of more than 150 drugs in influent wastewater samples and to evaluate their temporal variations in a period of four years (daily, weekly, monthly and seasonally) in order to have instantaneous information on changes in drug use trends during the economic crisis in Greece.

2. METHOD DESCRIPTION

2.1 Sample Preparation

Wastewater samples were collected in four different periods: December 2010, April 2011 April 2012 and March 2013 from the WWTP of Athens.

Samples (50 mL) were filtered through glass fibre to remove solid particles before solid phase excitation (SPE) procedure and acidified at pH 2.5 using HCl 1M. Appropriate concentrations of the internal standards were added to the water samples and SPE on Strata –X and Strata –XC cartridges was applied. The SPE procedure for Strata-X and for Strata-XC was derived from our previous work for the determination of illicit drugs and pharmaceuticals. Strata-X (200 mg/6mL) cartridges were rinsed with 6 mL methanol and 6 mL ultrapure water, under gravity. During sample pretreatment, 1 mL EDTA (5% w/v) was added, together with deuterated internal standards. Samples were percolated through cartridges and they were washed using 6 mL ultrapure water and dried for 1 h. Analytes were eluted with 2×3 mL of methanol. Conditioning of the Strata XC (200 mg, 6 mL) cartridge was executed under gravity with 6 mL of methanol and 6 mL of acidified ultrapure water (adjusted to pH 2.5 using HCl 1M). Next, samples (50 mL) were loaded also under gravity. Before elution of adsorbed analytes, the cartridge was washed with 3 mL of ultrapure water, pH 2.5, and subsequently dried under vacuum for 1 h. The elution was performed with 2×3mL of 2%(v/v) NH₃ in methanol (pH~10). Evaporation, reconstruction and filtering step of the extract are the same with both sorbents. The eluate was evaporated and the dried residue was reconstituted in 500 μL of a mixture of 25% (v/v) MeOH and 75% ultrapure water (0.05% v/v HCOOH), followed by a filtering step onto a 0.2 mm RC syringe filter. LC-MS/MS analysis followed.

2.2 LC-MS/MS

Liquid chromatography was performed with an Accela autosampler and gradient UHPLC pump (Thermo) and hyphenated to a TSQ Quantum triple-quadrupole mass spectrometer equipped with an electrospray ionization source. The mobile phase for positive ESI was ultrapure water with 0.01% v/v HCOOH and MeOH, whereas the one for negative ESI was a mixture of ultrapure water with 5 mM HCOONH₄, MeOH and ACN.
3. RESULTS

The proposed method for wastewater provided absolute recoveries between 50% (norephedrine, thiopental and SGN) and 113% (Topiramate) and slightly worse, but precise, recoveries, for cannabinoids (10% for cannabidiol to 20% for 11-nor-Δ9-THC acid), metformin and simvastatin (10%), mefenamic acid (18%), atrovastatin (22%), gemfibrozil (35%), ranitidine (41%) and pentobarbital (137%). Table 1 shows the overall performance of the methods for wastewater and the Figure 1 shows the trends of drug of abuse and pharmaceuticals in a long term period.

Table 1: Summary of validation results for LC-MS/MS methods for wastewater analysis.

<table>
<thead>
<tr>
<th>Recoveries</th>
<th>Number of compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>80-120%</td>
<td>115 compounds/160</td>
</tr>
<tr>
<td>50-79%</td>
<td>32 compounds/160</td>
</tr>
<tr>
<td>&lt; 50%</td>
<td>13 compounds/160</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>method LODs</th>
<th>Number of compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1ng/L</td>
<td>4 compounds/160</td>
</tr>
<tr>
<td>≤10ng/L</td>
<td>99 compounds/160</td>
</tr>
<tr>
<td>≤155ng/L</td>
<td>57 compounds/160</td>
</tr>
</tbody>
</table>

| RSD% (n=8)  | For all the compounds ≤22% |

Figure 1 shows...
Figure 1: Trends in discharge of pharmaceuticals, controlled psychoactive and illicit drugs as revealed from the influents of analysis of WWTP of Athens, Greece, in a term period of 2010, 2011 and 2012.
4. CONCLUSIONS

An advanced analytical methodology which was successfully validated and based on the use of LC-(ESI) MS/MS has been applied for the simultaneous determination of more than 150 compounds in influent wastewater. The results of an extensive four year monitoring allow better insight of usage trend of a wide range of compounds including pharmaceuticals, controlled psychoactive and illicit drugs. These monitoring data also provided useful information about the removals and the loads. It is notable that during the economic crisis in Greece, between 2010 and 2012, there is a significant increase of antidepressants and antipsychotics, as well as some specific illicit drugs as methamphetamine and methadone. To our knowledge, this is the first study reporting such data in that long term period.

REFERENCES