A nine-year study on the temporal changes of licit and illicit drug use patterns as revealed from the chemical analysis of influent wastewater from the wastewater treatment plant of Athens

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Abstract
Daily flow-proportional composite influent wastewater was collected from the Wastewater Treatment Plant (WWTP) in Athens during a nine-year sampling campaign (2010-2019). Analytes in influent wastewater are markers of the consumption and the exposure of the chemicals in the population. Wastewater samples were cleaned up and enriched 200 times using a generic solid phase extraction protocol based on a mixture of four sorbent materials capable of retaining a wide-range of analytes. Extracts were analyzed by a wide-scope targeted LC-QTOFMS and by highly-sensitive LC-MS/MS methods for the determination of pharmaceuticals such as antidepressants, anxiolytics, antipsychotics, antibiotics, antiepileptics, analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), diuretics, antihypertensives, antiulcer, and steroids as well as the main illicit drugs and their metabolites among others. Furthermore, the daily consumption of the substances was back-calculated with the aim of revealing trends in the use patterns of the compounds and associate the changes with socioeconomic phenomena, law enforcements and financial indicators.
Keywords: wastewater epidemiology, use patterns, socioeconomic changes, illicit drugs, pharmaceuticals, LC-MS/MS, LC-QTOFMS, wide-scope screening

1. Introduction

Wastewater treatment plants (WWTP) receive thounsands of substances daily in their facilities. The concentration levels of the chemicals contain informaton about the consumption and/or exposure of the population to these chemicals. Wastewater-based epidemiology (WBE) is the new research field that investigates the occurrence of emerging contaminants in influent wastewater and transforms the concentration levels in useful metrics for the society **(Castiglioni et al., 2006)**. One of the major applications of WBE is the determination of pharmaceuticals and illicit drugs in the influent of WWTP, which provides data on the amount and type of drugs used by a population. These data are used to back-calculate the mass loads of the parent drugs and subsequently to estimate the drug use, provided that the excretion patterns and drug metabolism are available. The main advantages of calculating the loads by analyzing the influent wastewaters is that it allows detection of changes in the patterns of pharmaceuticals and illicit drugs. Moreover, it offers the facility to compare data among different time sets and correlate them with socioeconomical factors **(Thomaidis et al., 2016)**. The objective of the study was to investigate the trends in drug consumption of various classes of pharmaceuticals (e.g. antidepressants, antipsychotics, gastric and ulcer drugs etc) during and after the financial crisis for years 2010-2019.

2. Material and Methods

2.1. Wastewater treatment plant

The Psyttalia WWTP is the third biggest in Europe and is designed for 5,200,000 population equivalents. The residential population connected to the WWTP based on official census in 2011 was 3,700,000 and the average wastewater flow is approximately 730,000 m^3/day. The WWTP of Athens is designed with primary sedimentation, activated sludge process with biological nitrogen and phosphorus removal and secondary sedimentation. Due to its size, analysis of wastewater gives a representative overview of the lifestyle and the health status of the population of Athens.

The sampling took place for at least one week of March in years 2010-2019. More specifically, samples were collected from 5th of December to 11th of December 2010 (7 samples) from 3rd of April to 10th of April 2011 (8 samples), from 2nd of April to 9th of April 2012 (8 samples), from 6th of March to 16th of March 2013 (11 samples), from 11th of March to 18th of March (8 samples), from 4th to 11th March 2015 (8 samples), from 16th to 23rd March 2016 (8 samples), from 8th to 14th March 2017 (7 samples), from 14th to 20th March 2018 (7 samples) and from 13th to 19th March 2019 (7 samples).

2.2. Sample preparation and Instrumental analysis

A generic sample preparation that followed enabled the sample clean-up and enrichment for a wide range of compounds with broad physicochemical properties. The four-layer solid-phase extraction (SPE) cartridge and the elution with both basic and acidic solutions allowed the extraction of neutral, aromatic, acidic, basic and polar analytes **(Kern et al., 2009)**. 200 times pre-concentration was applied to achieve adequately low limits of detection (LOD) of the analytes in wastewater.

Instrumental analysis was performed with a Thermo UHPLC Accela system connected to a TSQ Quantum Access triple quadrupole mass spectrometer from Thermo Electron Corporation (San Jose, CA, USA) equipped with an electrospray ionization source (Thermo Ion Max) in both positive and negative modes. Chromatographic separation was achieved on an Atlantis T3 C18 (100 mm × 2.1 mm, 3 μm) column from Waters Corporation (Milford, MS, USA) at a constant flow rate of 100 μL min^{-1}. The mobile phase, the gradient and rest instrumental settings can be found at Thomaidis et al., 2016. To capture substances not included in the LC-MS/MS target lists, extracts were also analyzed in an UHPLC system (UltiMate 3000 RSLC, Thermo Fisher Scientific, Germany) coupled to a QTOF-MS (Maxis Impact, Bruker Daltonics, Bremen, Germany). Two separate reversed-phase chromatographic runs were performed for positive and negative ESI mode using a Thermo Acclaim RSLC 120 C18 column (2.1×100 mm, 2.2 μm). Data were acquired through two different scan modes with a scan rate of 2 Hz and a mass range of 50-1000 Da. The first scan mode was a Data Independent Acquisition (DIA), termed broad-band Collision Induced Dissociation (bbCID), in which low collision energy (4 eV) provided a full scan spectrum (MS) and high collision energy (25 eV) provided a spectrum, in which all ions were fragmented (bbCID MS). The second scan mode was a Data Dependent Acquisition (DDA), termed AutoMS, which provided a full scan spectrum (MS), in which the five most abundant precursor ions were isolated and fragmented resulting in their HRMS/MS spectra. More details about the method can be found in Alygizakis et al.

3. Results

Compounds were divided in eight categories: Antibiotics, Antiepileptic drugs, Antihypertensive drugs, Antilipidemics and diuretics, Antipsychotics, Benzodiazepines and antidepressants, gastric and ulcer drugs, illicit drugs and Nonsteroidal anti-inflammatory drugs (NSAIDs). Psychoactive drugs is directly associated with mental illnesses in the population. A high increase in the use of psychoactive drugs was detected between 2010 and 2014, especially for antipsychotics (35-fold), benzodiazepines (19-fold), and antidepressants (11-fold). However, for period 2015 to 2019, a slight decrease in use of psychoactive drugs was observed. Consumption of all investigated compounds in the category showed a decreasing trend. The decrease observed was not equal to the pre-crisis increase observed during the period 2010-2014. This means that the use of all psychoactive drugs remains still at high concentration levels (especially for citalopram and several benzodiazepines).

Notable trend was observed for antibiotics and NSAIDs. Both classes of therapeutic drugs showed a decrease for period 2010-2014, likely related to the reduction in drug expenditure applied in public health and change in the prescription strategy (case of mefenamic acid for which an almost 28-fold decrease was observed for 2010-2014). The trend was reversed for period 2014 to 2019 with a disproportional increase for antibiotics (+89 %), which exceeded the levels of 2010. All NSAIDs increased except for mefenamic acid, the trend of which continued to decrease for the period 2014 to 2019. Antihypertensive drugs and diuretics showed a slight increase (less than 10 %), whereas antiepileptic drugs and gastric and ulcer drugs did not show any remarkable trend. Finally, illicit drugs showed an overall decrease for the period 2015-2019 with the exception of cannabis.

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