Target and suspect screening approaches for the identification of new psychoactive substances in raw wastewater samples from Athens by LC-QTOF-MS

Diamanti K.[1], Thomaidis N.[1]

[1]National and Kapodistrian University of Athens, Department of Chemistry, Laboratory of Analytical Chemistry, Panepistimiopolis Zografou, 15771, Athens, Greece

E-mail: ntho@chem.uoa.gr

EXTENDED ABSTRACT

Over the last fifteen years, scientific attention was attracted by wastewater-based epidemiology (WBE), an innovative approach that calculates the drug consumption in a community by analyzing specific biomarkers in wastewater. It relies on the fact that almost everything that population consumes is excreted as parent compound or metabolite in urine and faeces and ends up in the sewer network *(Castiglioni 2016)*. WBE has widely been applied for the estimation of illicit drug use *(Thomas et al. 2012; Thomaidis et al. 2016)* and has recently been expanded to the challenging detection of new psychoactive substances (NPS) *(Borova et al. 2015; Kinyua et al. 2015)*. These compounds mimic effects of illicit drugs and are produced to evade law enforcement by introducing slight modifications to chemical structures of controlled illicit drugs *(Kinyua et al. 2015)*.

Advanced analytical techniques are required to identify these substances in raw wastewater because of their low concentration and the complexity of the wastewater matrix. Liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) allows the wide-scope screening of many parent substances, metabolites and transformation products with an acquisition of accurate-mass full spectrum data. These data can be used for retrospective analysis in order to investigate new substances or unexpected and not searched ones in wastewater, even after years, without additional analysis of the samples *(Gago Ferrero et al 2015)*.

This study reports qualitative screening of new psychoactive substances in raw wastewater samples from the main wastewater treatment plant of Athens in Psyttalia using LC-QTOF-MS. The samples were collected for 8 consecutive days on March 2014, 2015, 2016, 2017 and a solid-phase extraction technique using mixed bed multilayer cartridges containing different extraction sorbents was used for sample clean-up and pre-concentration. Two separate reverse phase chromatographic runs were performed for positive and negative ESI mode. Data were acquired through broad-band Collision Induced Dissociation (bbCID) mode that provided both MS and MS/MS spectra simultaneously, with a mass range of 50-1000 Da and a scan rate of 2 Hz.

In target screening approach, an in-house database of approximately 200 new psychoactive substances was used. The database contained precursor ions, retention time, adducts, in-source fragments and MS/MS fragments. The raw data were analyzed with Bruker's TASQ Client 1.0 and DataAnalysis 4.3. Thresholds such as retention time ±0.2 min, mass accuracy ±2.5 mDa and isotopic fitting ≤ 200 mSigma were used for the identification of the compounds. In order to confirm the compounds, MS/MS fragments were examined, as well as adducts and in-source fragments.

In suspect screening approach, a database of approximately 500 new psychoactive substances was built using only exact mass as a priory information. The raw data were analyzed with Bruker's TargetAnalysis 1.3 and DataAnalysis 4.3. It was assumed that all suspect compounds produce $[M+H]^+$ or $[M-H]^-$ when they ionized by the ESI source. For the tentative identification of the compounds, specific criteria in area, intensity, signal-to-noise ratio, mass accuracy and isotopic fitting were evaluated. Then, the experimental retention time was compared with the predicted retention time from an in-house QSRR retention time prediction model *(Aalizadeh et al 2016)*. Some of these tentative candidates were confirmed in a better confidence level by the presence of characteristic adduct ions and MS/MS fragments.

Following the aforementioned procedures, few of the investigated compounds were identified, as they fulfilled all the criteria. The first results indicate an occasional use of NPS in the population of Athens over the years. Specific substances, such as 2-phenethylamine and PMMA are detected every year, while MDAI, methoxetamine, MPPP are detected occasionally. The phenethylamine 2 C-D, the synthetic cannabinoid 5-Cl-AB-PINACA, the piperazine derivative MBPZ are interesting examples of NPS that are detected for the first time in raw wastewater.

References:

1. Aalizadeh R., Thomaidis N. S., Bletsou A., Gago-Ferrero P., "Quantitative Structure-Retention Relationship models to support non-target high resolution mass spectrometric screening of emerging contaminants in environmental samples", *Journal of Chemical Information and Modeling*, vol. 56 (7), pp 1384-1398, **2016**

2. Borova V. L., Gago-Ferrero P., Pistos C., Thomaidis N. S., "Multi-residue determination of 10 selected new psychoactive substances in wastewater samples by liquid chromatography–tandem mass spectrometry", *Talanta*, vol. 144, pp 592-603, **2015**
3. Castiglioni S., "Assessing illicit drugs in wastewater - Advances in wastewater-based drug epidemiology", European Monitoring Centre for Drugs and Drug Addiction, **2016**, ISBN: 978-92-9168-857-9
4. Gago Ferrero P., Schymanski E., Bletsou A., Aalizadeh R., Hollender J., Thomaidis N., "Extended suspect and non-target strategies to characterize emerging polar organic contaminants in raw wastewater with LC-HRMS/MS", *Environmental Science and Technology*, vol. 49 (20), pp 12333-12341, **2015**
5. Kinyua J., Covaci A., Maho W., Mccall A.-K., Neels H., van Nuijs A. L. N., "Sewage-based epidemiology in monitoring the use of new psychoactive substances: Validation and application of an analytical method using LC-MS/MS", *Drug Testing and Analysis*, vol. 7 (9), pp 812-818, **2015**
6. Thomaidis N. S., Ferrero P. G., Ort C., Maragou N. C., Alygizakis N. A., Borova V. L., Dasenaki M. E., "Reflection of socio-economic changes in wastewater: licit and illicit drug use patterns", *Environmental Science and Technology*, vol. 50 (18), pp 10065–10072, **2016**
7. Thomas K. V., Bijlsma L., Castiglioni S., Covaci A., Emke E., Grabic R., Hernandez F., Karolak S., Kasprzyk-Hordern B., Lindberg R. H., de Alda M. L., Meiergohann A., Ort C., Pico Y., Quintana J. B., Reid M., Rieckermann J., Terzic S., van Nuijs A. L. N., de Voogt P., "Comparing illicit drug use in 19 European cities through sewage analysis", *Science of Total Environment*, vol. 432, 432-439, **2012**

Keywords: NPS, WBE, LC-QTOF-MS, target screening, suspect screening