A High-throughput 96-well Elution Protocol for the Quantification of Psychoactive Substances in Influent Wastewater as an Alternative for Traditional Solid-Phase Extraction.

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Introduction

- There is an increasing need for high resolution temporal data in wastewater-based epidemiology (WBE), e.g. Sewage analysis CORe group (SCORE) Monitoring campaign uses 7 datapoints per year
- Due to the complex wastewater matrix, it is crucial to have sensitive analytical procedures available
- To enhance the usefulness of WBE as a complementary epidemiological source, there is a growing pressure to analyse more compounds, more locations and more samples
- Additionally, the demand for more green chemistry is rising
  → Objective: development of a more efficient and high-throughput sample preparation procedure

Methods

- A high-throughput method based on 96-well Oasis MCX solid-phase extraction (SPE) was developed in accordance to the European Medicine Agence (EMA) guidelines with minor adjustments (Figure 1)
  - LC-MS/MS: Waters Atlantis T3 (150 x 2.1 mm, 3 μm), Agilent 6495 Triple Quadrupole MS/MS
  - Mobile phase A: H₂O + 0.1% formic acid (v/v), Mobile phase B: MeOH + 0.1% formic acid (v/v)
- External quality control was performed through participation in interlab study by SCORE
- The validated method was applied to influent wastewater samples (n=496) of Leuven, Belgium to analyse the use of amphetamine, cocaine (through benzoylecgonine) and 3,4-methylenedioxymethamphetamine (MDMA) from September 2019 to April 2022.

Results

- Validation was successful for 28 parent drugs and metabolites of antidepressants (e.g. mirtazapine), opioids (e.g. tramadol) and stimulants (e.g. amphetamine) (Figure 3)
- Lower limit of quantification (LLOQ) ranged from 1 - 30 ng/L (except tramadol, 100 ng/L)
- Highest opioid and antidepressant population normalized mass load (PNML) were measured for tramadol (1096 mg/day/1000p) and O-desmethylvenlafaxine (754 mg/day/1000p) respectively
- Capable of monitoring high resolution temporal trends in the use of stimulants and pharmaceuticals (see Figure 2 for stimulants)

Figure 1. Comparison between traditional SPE and 96-well SPE

Figure 2. Temporal analysis (n=496) of amphetamine, cocaine (through benzoylecgonine) and 3,4-methylenedioxymethamphetamine (MDMA) using 96-well extraction. Weekly averages, based on three data points per week (Monday-Wednesday-Saturday) were used. Teal color shows an interpolated week. The population size was estimated based on mobile phone data. Methamphetamine was also analysed, but values were below LLOQ.

Figure 3. List of included parent drugs and metabolites

Conclusion

- The 96-well SPE has demonstrated its ability as an alternative for the traditional SPE method
- The validated method which employed a 96-well solid-phase extraction process proved to be more efficient than the traditional SPE, requiring less time, sample volume and organic solvents (Figure 1)
- The method was applied to 496 samples, showing its capability of analysing a large amount of samples and thereby scaling up the spatio-temporal analysis possibilities