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-troughput sample preparation procedure**

Methods

- A high-troughput 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: H_20 + 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.

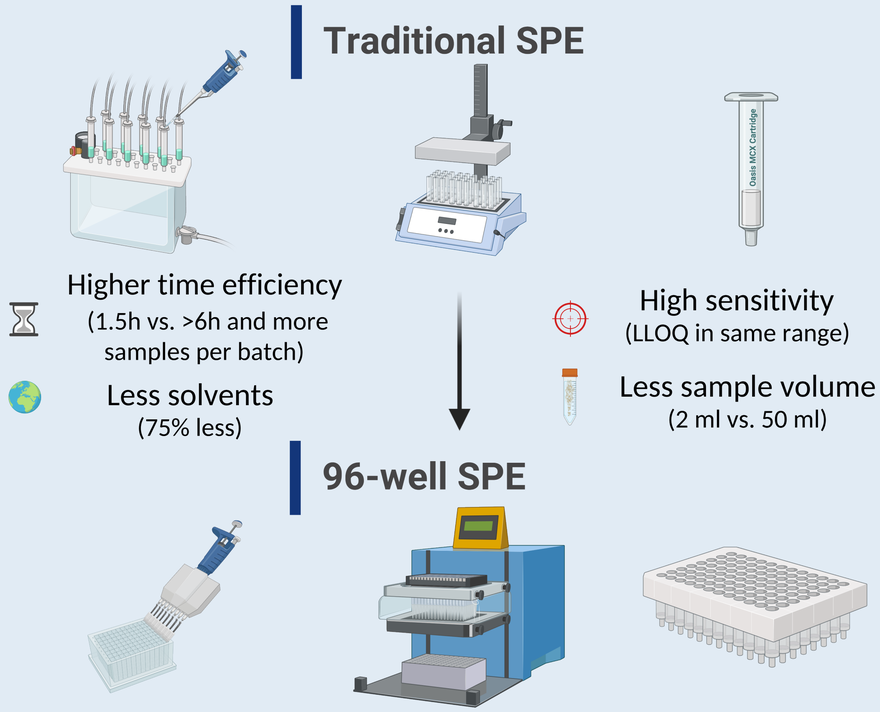

Figure 1. Comparison between traditional SPE and 96-well SPE

Results

- **Validation was succesful** 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*)

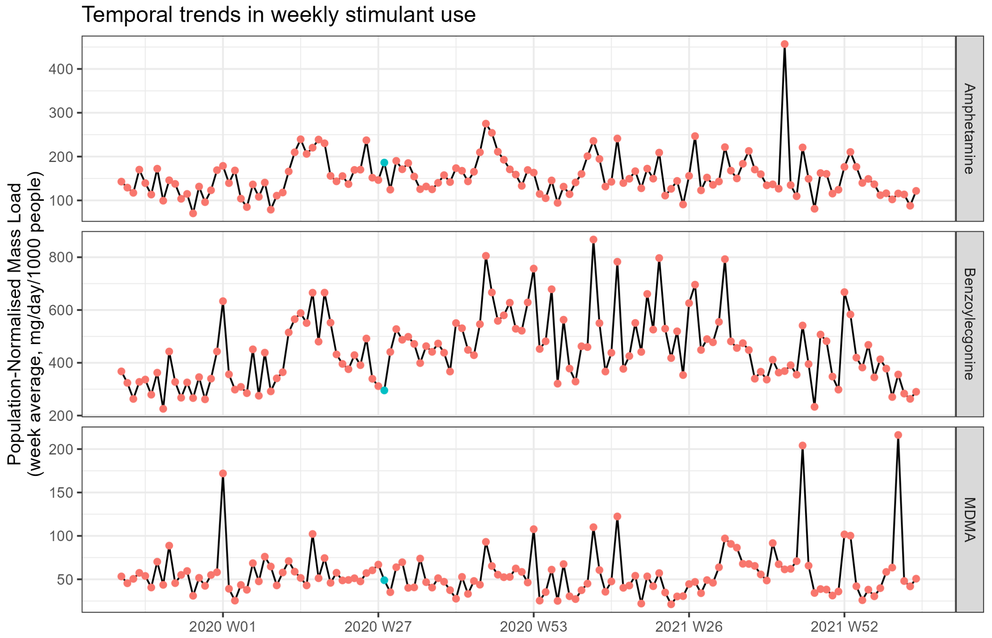

Figure 2. Temporal analysis (n=496) of amphetamine, cocaine (through enzoylecgonine) and 3,4-methylenedioxymethamphetamine (MDMA) using 96-well extraction. Weekly averages, based on three data points per week (monday-wednesday-saturday) were used. Teal colour shows an interpolated week. The population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

Opioids
2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)
Codeine
Dihydrocodeine
Hydromorphone
Methadone
Morphine
Normorphine
Noroxycodone
Nortilidine
O-desmethyltramadol (ODT)
Oxycodone
Tilidine
Tramadol
Antidepressants
Amitriptyline
Bupropion
Bupropion-OH
Citalopram
M-chlorophenyl piperazine (MCPP)
Melitracen
Moclobemide
Norcitalopram
Normirtazapine
O-desmethyl venlafaxine (O-DMV)
Trazodone
Venlafaxine
Stimulants
3,4-methylenedioxy_ methamphetamine (MDMA)
Amphetamine
Benzoylecgonine
Methamphetamine

Figure 3. List of inlcuded 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