Evaluation of the Exposome:
Non-targeted screening analysis of environmental contaminants in human urine by liquid chromatography coupled to high resolution mass spectrometry

WWW.ISA-LYON.FR

Aurélie Cortéjade, Agneta Kiss, Cécile Cren, Emmanuelle Vulliet, Audrey Buleté

Université de Lyon, Institut des Sciences Analytiques, UMR5280 CNRS, Université Lyon 1, ENS-Lyon, 5 rue de la Doua, 69100 Villeurbanne, France

Introduction & objectives

The impact of the environment on human health has been conclusively demonstrated. In recent decades, scientists have shown that many chronic diseases are related to our environment. In this context, a new term was born in 2005: the Exposome. It corresponds to all types of exposures humans are subjected throughout their lives via lifestyle, diet and social environment, as well as the body responses to these exposures. The exposures the man is facing are numerous and all environmental contaminants present in everyday life are part of the Exposome. So the concept of Exposome highlights the need to develop measurement methods to evaluate human exposures. Furthermore, the Exposome is an dynamic concept that evolves over time and space. Therefore, it is necessary to rapidly establish sensitive routine measurement methods.

In this context, a method was developed on urine and on a LC-QqToF instrument to detect contaminants, metabolites and degradation products of known contaminants in contact to man in daily routine by a comprehensive approach. The developed analytical strategy consists in a broad screening of the urine based on the exact monoisotopic mass of the environmental contaminants, their metabolites and degradation products contained in urine. The urine appears to be an advantageous biological matrix for studying the Exposome: it is easy to obtain (non-invasive, readily available) and includes a large number of endogenous and exogenous metabolites.

Materials & methods

Sample preparation

Preparation of 4 types of urine sample :

Crude urine (without sample preparation)

Concentration of urine by SPE on Oasis® HLB

10 0mL of urine
Elution with 4 mL of methanol
Evaporation of elution solvent

Liquid-liquid extraction with ethyl acetate

4 mL of urine
16 mL of ethyl acetate
Evaporation of organic phase

Liquid-liquid extraction with methyl tert-butyl ether

8 mL of urine
20 mL of MTBE
Evaporation of organic phase

Extracts dissolved in 500 µL of water
Injection in UHPLC-MS/MS

Analysis : UHPLC-QqToF

System:

UHPLC Ultimate™ 3000 (Thermo®)

Separation:

ESI+

Column: XSelect CSH C18 column (2.1 x 100mm; 3.5µm) (Waters®)

Mobile phase: ACN (0.1 formic acid, 10mM formate ammonium)/ Water (0.1 formic acid, 10mM formate ammonium)

ESI-

Column: Kinetex C18 column (2.1 x 100mm; 2.6µm) (Phenomenex®)

Mobile phase: ACN (0.1% acetic acid) / Water (0.1% acetic acid)

Acquisition:

A quadrupole-time-of-flight mass spectrometer MicrOTOF-Q II™ (Bruker Daltonics®)

with an electrospray ionization source

Acquisition parameters:

Mode	ESI+	ESI-
m/z range	50-600	50-1000
Capillary	+2500V	-3500V
Dry gas	12L/min	12L/min
Nebulizer	3 Bar	3 Bar
Source temperature	250°C	250°C

Results & discussions

Choice of the most appropriate sample preparation

Total ion current chromatograms (TIC) in ESI + and ESI- after injection of each sample :
- Crude urine
- Concentrated urine
- Compounds extracted in urine by liquid-liquid extraction with ethyl acetate
- Compounds extracted in urine by liquid-liquid extraction with methyl tert-butyl ether

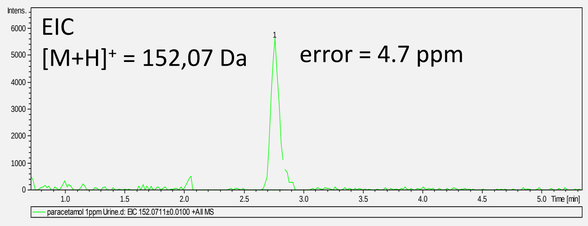
The **urine concentrated by SPE** contains more compounds, more information than other samples

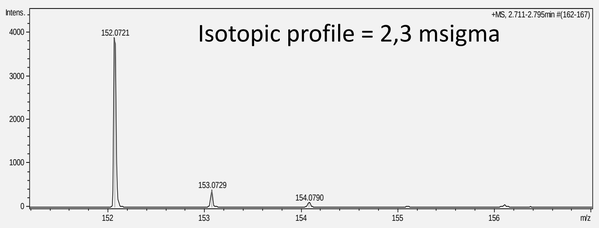
Processing data

Targeted method

1) Search of various contaminants such as human drugs or surfactants in urine, as well as known metabolites (from literature data), then generation of **Extracted Ions Chromatograms** (EIC)
Criteria : exact mass (error < 5 ppm) and isotopic profile (< 30 msigma)

Example : Paracetamol ($C_8H_9NO_2$)





2) **Fragmentation** of ion $[M+H]^+$ = 152,07 Da in sample and standard solution of paracetamol at 15 eV and 20 eV
Comparison of the fragmentation spectrum

→Same retention time and fragment ion m/z = 110.05 Da
→ Same ratio between precursor ion and fragment ion at 2 collision energies

➔ Hypothesis of identification of paracetamol in sample urine

Voluntary urine donor claims not to have consumed paracetamol.
Where does it come from? What are the possible sources of contamination? Pollution of water? Food?

Non-targeted method

1) **Comparison** of m/z between sample and data bank as DrugBank or HMDB
→ Obtaining a list of potential compounds present in sample

Data bank	DrugBank		HMDB	
ESI mode	+	-	+	-
Number of compounds	234	128	218	143

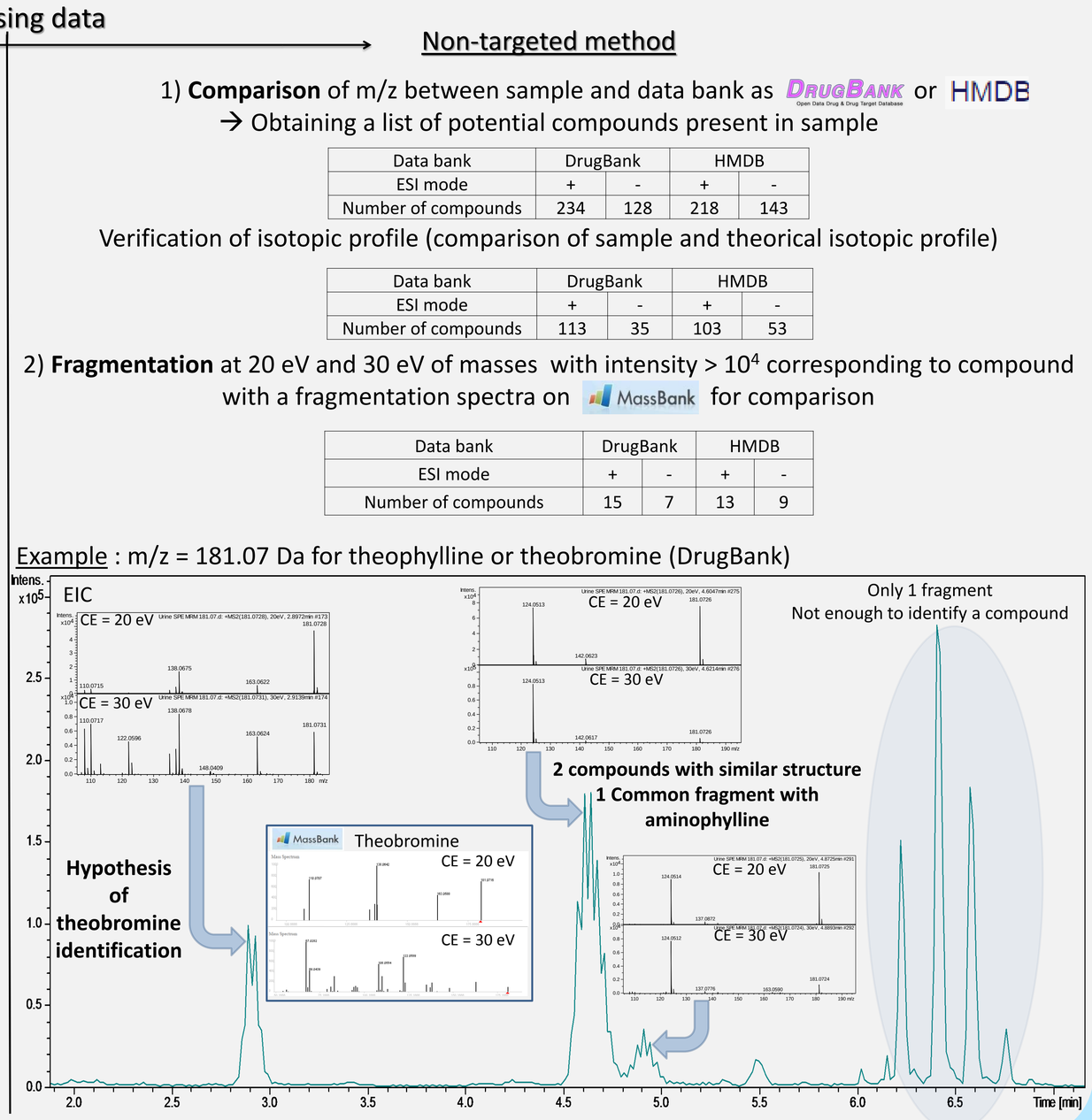
Verification of isotopic profile (comparison of sample and theorical isotopic profile)

Data bank	DrugBank		HMDB	
ESI mode	+	-	+	-
Number of compounds	113	35	103	53

2) **Fragmentation** at 20 eV and 30 eV of masses with intensity > 10^4 corresponding to compound with a fragmentation spectra on MassBank for comparison

Data bank	DrugBank		HMDB	
ESI mode	+	-	+	-
Number of compounds	15	7	13	9

Example : m/z = 181.07 Da for theophylline or theobromine (DrugBank)



Conclusion

Two complementary screening approaches were realized to evaluate human exposures: targeted and non-targeted. A large number of data was obtained. Different compounds were detected in urine samples by using one or more identification criteria. The critical step of this study was the processing data which required the use of databases in non-targeted method.
The implementation of this tool to identify environmental contaminants in human urine associated with statistical and bioinformatics studies, contribute greatly to the understanding of the causal relationships between diseases and environmental factors. But the study of the understanding of the Exposome requires multidisciplinary approach and disciplines that are exposure science, epidemiology, molecular biology, analytical chemistry.

Contact e-mail: audrey.bulete@isa-lyon.fr