Endocrine disrupting chemicals are substances that can interact with the endocrine system causing alteration of the endocrine homeostasis and potentially lead to adverse health effects. They have been linked to numerous endocrine diseases such as reproductive disorders, infertility, hormone-dependent cancers, obesity, diabetes, neuro-developmental disorders, ...

Phthalates, Parabens and Benzophenone-3 (BP3) have endocrine disrupting properties and they are high production volume (HPV) chemicals. More than 75% of general population’s urines were found positive for most of them leading to an important public health issue.

These compounds are rapidly excreted from the human body, their analysis in urine provides information about recent exposure. To assess human exposure and investigate potential endocrine disruptive health injuries, we developed an Ultra High Pressure Liquid Chromatography coupled to Tandem Mass Spectrometry analytical method for simultaneous measurement of phthalate metabolites, parabens and BP3 in human urine.

### MATERIAL AND METHOD

#### Hydrolysis and Extraction

**Hydrolysis**

- **Urine**: 3 ml
- **Internal standard**: 1 ml (10 mM HAc / ACN HAc 0.1% (A / B)
- **Buffer**: 750 mL
- **Glucuroconjugate treatment**: 10 mM HAc 10 ml / 1 ml (A / B)
- **E. coli hydrolysis**: 10 mM HAc 10 ml / 1 ml (A / B)
- **No hydrolysis**: Free
- **Hydrolysis over night incubation at 37°C**

**Glucuroconjugate treatment**

- **Buffer**: 10 ml / 1 ml (A / B)
- **Glucuro-conjugated**: 10 mM HAc 10 ml / 1 ml (A / B)
- **Escherichia coli**: 10 mM HAc 10 ml / 1 ml (A / B)
- **Phosphate buffer**: 10 ml / 1 ml (A / B)
- **Hydrolysis over night incubation at 37°C**

**Validation**

- **Total error method approach E-noval software (Arenda)**
- **Calibration points in duplicate during 3 days**
  - From 0.5 to 200 µg/L (most compounds)
  - From 2 to 800 µg/L (MP, BP3)
- **Validation points in triplicate during 3 days**
  - From 0.2 to 200 µg/L (most compounds)
  - From 0.2 to 800 µg/L (MP, BP3)

#### Chromatogram

**UPLC-Acquity (Waters)**

- **Mobile phase**: H2O HAc 0.1% / ACN HAc 0.1% (A / B)
- **Column**: Kinetex 1.7 µm Phenyl-Hexyl 100A 100x2.1 mm (Phenomenex)
- **Flow**: 0.55 mL/min, gradient elution mode (from 91% to 20% A), 35°C
- **Injection volume**: 5 µL
- **Run time**: 20 min

**Tandem MS Quattro Premier XE (Waters)**

- **ESI** (−3kV) except for BP3, ESI + (4.0 kV)
- **2 MRM studied by analyte**

#### Results

**E. coli validation results**

The acceptance limits were set at 30% for concentration upper of 2 µg/L for EP, PP, MnBP, MEHP, 5-oxo-MEHP, 5-OH-MEHP, MEHP, MBzP, BP3 and 5 µg/L for MP, MBP and BP. They were set at 50% for concentration from LOQ to 2 µg/L for EP, PP, MnBP, MEHP, 5-oxo-MEHP, 5-OH-MEHP, MEHP, MBzP, BP3 and 5 µg/L for MP, MBP and BP.

**CONCLUSION**

We developed a sensitive method for determination of seven phthalate metabolites, four parabens and benzophenone-3. This method was fully validated according the total error method approach. The three enzymatic conditions were validated. This method can now be used for human exposure monitoring and to investigate potential health injuries of these endocrine disrupting chemicals.