Simultaneous determination of phthalate metabolites, parabens and benzophenone-3 in human urine by ultra-high-pressure-liquid-chromatography tandem mass spectrometry

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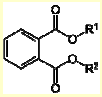
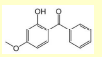
INTRODUCTION

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 numbers endocrine diseases such as reproductive disorders, infertility, hormone-dependant cancers, obesity, diabetes, neuro-developmental disorders,…

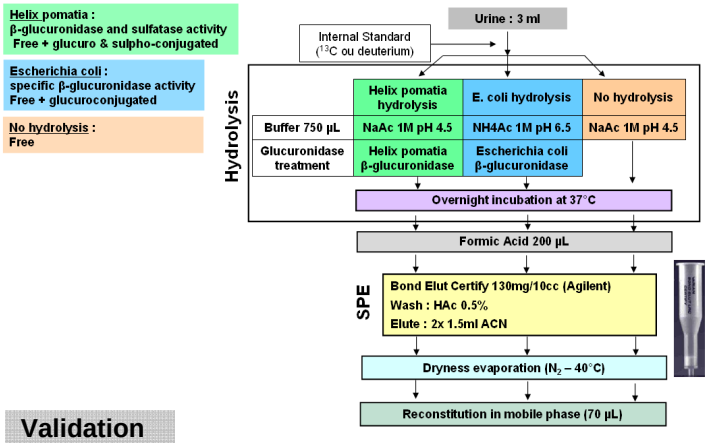
Phthalates, Parabens and Benzophenone-3 (BP3) have endocrine disrupting properties and they are high production volume (HPV) chemicals. More than 75% of general population's urines were found positive for most of them leading to an important public health issue

These compounds are rapidly excreted from 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.

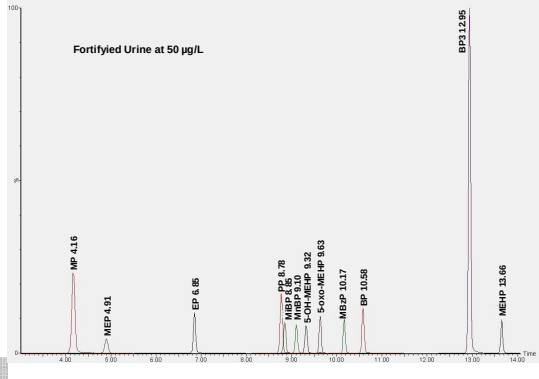
	USES	PARENT PRODUCT	BIOMARKER OF EXPOSURE	PHASE II METABOLISM
PARABENS 	Antimicrobial conservator (cosmetics, sunscreens, foodstuffs and pharmaceu-tical preparations)	Methylparaben Ethylparaben Propylparaben n-Butylparaben	Methylparaben (MP) Ethylparaben (EP) Propylparaben (PP) n-Butylparaben (BP)	Glucuro and sulfo-conjugated
PHTHALATES 	Plasticizer (PVC, construction materials, solvents, lubricants, personal care products, textiles, food contact materials)	Diethyl phthalates Di-n-butyl phthalate Di-iso-butyl phthalate Benzylbutyl phthalate Di-2-ethylhexyl phthalate	Monoethyl phthalate (MEP) Mono-n-butyl phthalate (MnBP) Mono-iso-butyl phthalate (MiBP) Monobenzyl phthalate (MBzP) Mono-2-ethylhexyl phthalate (MEHP) Mono-2-ethyl-5-hydroxyhexyl phthalate (5-OH-MEHP) Mono-2-ethyl-5-oxohexyl phthalate (5-oxo-MEHP)	Glucuro-conjugated
BENZOPHENONE-3 	UV-filter (sunscreens /soap)	Benzophenone-3	Benzophenone-3 (BP3)	Glucuro-conjugated

MATERIAL AND METHOD

Hydrolysis and Extraction



Chromatogram



Validation

Total error method approach E-noval software (Arlenda)

- **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)

LC-MSMS method

UPLC-Acquity (Waters) :

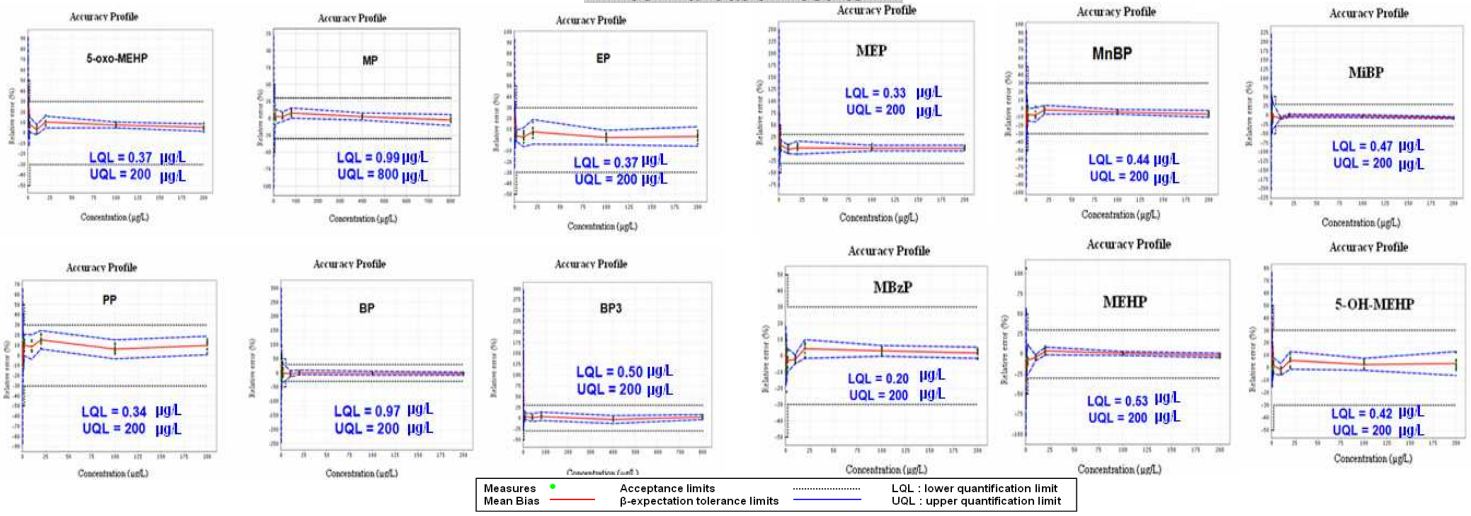
- **Mobile phase**: H_2O HAc 0.1% / ACN HAc 0.1% (A / B)
- **Column**:Kinetex 1.7u Phenyl-Hexyl 100A 100x2.1 mm (Phenomenex)
- **Flow**: 0.55 mL/min, gradient elution mode (from 91% to 20% A), 35°C
- **Injection volume**: 5 µL
- **Run time**: 20 min

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, MEP, 5-oxo-MEHP, 5-OH-MEHP, MEHP, MBzP, BP3 and 5 µg/L for MP, MiBP and BP. They were set at 50% for concentration from LOQ to 2 µg/L for EP, PP, MnBP, MEP, 5-oxo-MEHP, 5-OH-MEHP, MEHP, MBzP, BP3 and 5 µg/L for MP, MiBP 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.