IDENTIFICATION OF EMERGING CONTAMINANTS IN HUMAN URINE BY NON-TARGET SCREENING WITH LC-QTOFMS

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- **Emerging contaminants (ECs)** are compounds present in the environment for which there is a lack of chemical information but that are suspected to be harmful for humans and wildlife. Thus, there is an urgent need to identify and establish a set of **representative biomarkers** to assess the human exposure to ECs.
- **Human urine is a complex matrix**, most of the **ECs are present at trace levels (ng/mL)** and many types of contaminants are metabolized through different pathways and excreted via the urine as **metabolites**.
- **Non-target screening analysis** of human urine samples by high resolution mass-spectrometry is able to provide an **overview of the presence of ECs**. Since there are **no harmonized methods,** the obtained **results among different labs are not comparable neither reproducible**.
- The development of **reliable and comparable non-target/suspect screening workflows requires a complete set of QA/QC measures** in each single step of the workflow.
- **Quality assurance (QA)** is a set of activities or procedures which are adopted in a laboratory to ensure that all quality requirements will be fulfilled.
- **Quality control (QC)** refers to operational techniques and activities that are used to fulfil requirements for quality.

A universal QA/QC framework has been developed and applied to non-target screening analysis of human urine samples by LC-QTOFMS

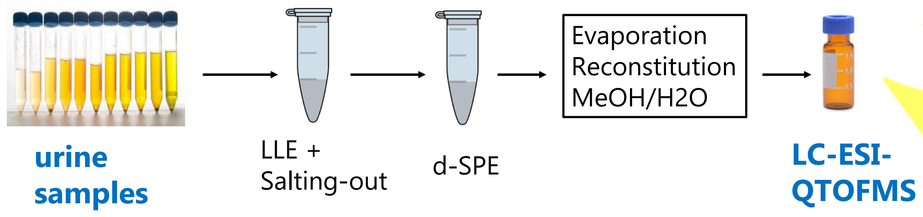
SAMPLE TREATMENT

- Urine contents salts, proteins and phospholipids that cause matrix-effects during the LC-QTOFMS analysis.
- Sample treatment must maintain a balance between maximal compound extraction and minimal matrix-effect.

1 Background contamination
- Limited use of plastic material
- High quality of solvents
- Baked glass labware

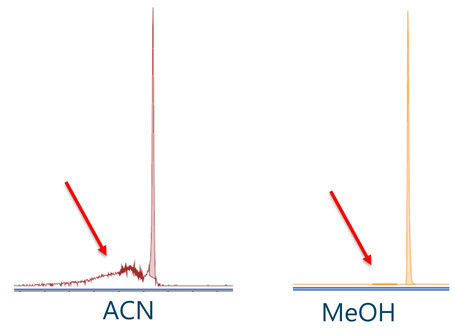
2 MIX Isotopic labelled IS
- Wide range of polarities
- Expected families of compounds
- Endogenous compounds
- Parent + Metabolites

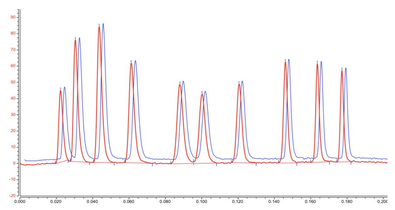
3 Optimization & Pre-validation method ⇨ Pooled-urine sample MIX IS
- Selectivity
- Extraction efficiency
- Universality
- Time
- Cost



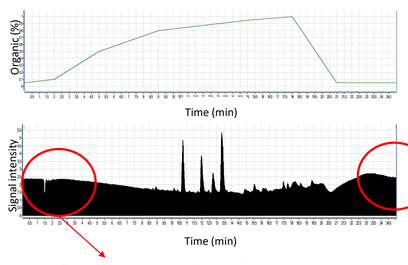
LIQUID CHROMATOGRAPHY

1 MIX-IS
- Selection of mobile phase composition based on peak intensity, adducts and shape
- Maximum resolution but keeping a soft chromatography gradient
- Check if retention time shifts inter-& intra-worklist exist
- Minimum volume of injection to avoid detector saturation and bad peak shapes
- Check if carry-over exists
- Check if overlapping of injections exits
- Test of the analytical column for background contamination





2 Visual inspection
- Pressure profile
- TIC: no compounds elution during the last 2 minutes of chromatogram
- Noise level



QA/QC

MS ANALYSIS

- MS scan: 50-1500 *m/z*
- MS/MS scan: 50-1400 *m/z*
- 4 Ions in MS automatically selected for MS/MS by abundance
- Collision energy: 20 eV
- Data storage: Centroid

1 Non-fortified controls
- Pooled-urine samples
- Solvent Blanks

2 MIX-IS fortified controls
- Procedural Blanks
- Pooled-urine samples
- MIX-IS

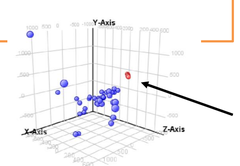
3 Others controls
- Mass calibration
- Duplicate samples
- Randomized worklist

DATA-ANALYSIS

1 Manual checking – MIX IS
- IS peaks intensities and mass accuracy in all samples
- Retention time shifts in samples and controls
- Matrix effect for IS in all samples

2 Suspect list
- Metabolite prediction
- Metabolites phase I & II
- MIX-IS
- Endogenous compounds
- High detection frequency compounds i.e. drugs

3 Feature extraction
- Aligment
- No Normalisation: high variability of urine composition
- No Blank substraction: ECs are omnipresent compounds
- Statistical analisys: Controls must be clusted



4 Compound identification
- Mass accuracy 5-10 ppm
- Coherence retention time/structure
- Isotopic pattern (ions accuracy+ relative abundance)
- Comparison of MS/MS spectra with databases
- Report quality of identification i.e. Schymanski´s scale (ES&T 2014, 48(4):2097-8)

Results

- More than 20 ECs were identified at identification levels 2 to 4 according to Schymanski´s scale. The achievement of higher levels of identification for many compounds was not possible owing to the lack of MS/MS data available for ECs.
- The highest detection frequencies (DF) are shown in the table:

COMPOUND GROUP	COMPOUND	DF (%)
Benzotriazole metabolite	Hydroperoxide-tolytriazole	100
Phthalate metabolite	Mono-iso-nonyl phthalate	100
Plasticizer	Bisphenol B	95
UV filter metabolite	[hydrox[y2-ethylhexyl 4-(dimethylamino)]benzoate	77

Conclusions

- Although many ECs present in human urine are not yet characterized, a good point to evaluate the whole non-target/suspect screening workflow may be the comparison with the obtained results by other laboratories.
- The establishment of a detailed list of QA/QC measures represents a good starting point for the harmonization of non-target/suspect screening methodologies used in human urine analysis.

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