Quality assurance and quality control of non-target screening of emerging contaminants in human urine by LC-HRMS

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1. Introduction

The concept of contaminants of emerging concern (CECs) refers to compounds that are not (yet) included in monitoring programs because they are new or their presence in the environment has not been elucidated or understood. However, they may have the potential to exhibit toxicity in humans and wildlife [1]. The impacts of human exposure to mixtures of chemicals are poorly understood, because biomonitoring campaigns do not include CECs. Therefore, there is an urgent need to establish a set of representative biomarkers to assess the human exposure to mixtures of CECs. Human urine is a complex matrix and the expected concentration for most of the CECs is at trace levels. In addition, presumably many types of contaminants are metabolized through different pathways and (partially) excreted through the urine. Non-target screening analysis of human urine samples by high resolution mass-spectrometry is able to provide an overview of the presence of CECs in the population. However, despite the attractive features of this novel strategy, it is facing a lack of harmonised methods that would permit obtaining comparable and high quality results.

Quality assurance (QA) is defined as a set of activities or procedures which are adopted in a laboratory to ensure that all quality requirements will be fulfilled, while quality control (QC) refers to operational techniques and activities that are used to fulfil requirements for quality [2]. To facilitate the development of reliable and comparable non-target/suspect screening workflows for the assessment of CECs in human urine by liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS), we will further develop a generic QA/QC framework.

2. Materials and methods

The achievement of an actual and representative fingerprint of CECs in human urine is a challenge that requires the establishment of proper QA/QC measures for each individual step of the workflow (Figure 1).

Figure 1: Non-target/suspect screening workflow. Adapted from Oberacher et al. [3,4].

QA/QC have been established considering their impact in both individual steps and the global workflow.

3. Results and discussion

3.1. Sample preparation

Sample treatment is a key step in the non-target/suspect screening analysis owing to the complexity of the challenges to be solved. One of the main issues is to get a balance between the effective removal of matrix interferences compounds and the improvement in the signal of the analytes of interest. Some components of urine can cause ion supression (i.e. phospholipids) or other matrix effects, so sample extraction must be non-selective to be able determine as many compounds as possible but also being efficient in the purification

of the sample. A set of labeled standards in a wide range of chemical properties must be added to the sample before the sample treatment. Furthermore, procedural blanks (spiked and non-spiked with mixtures of presumably relevant standards) will be processed in parallel. All labware will be tested for potential contamination of the most typical contaminants at analytical laboratories (i.e. plasticizers, phthalathes, flame retardants). Some of the crucial QA/QC measures will be presented and discussed.

3.2. Liquid chromatography (LC)

Since most analytical laboratories use reversed-phase LC, such stationary phases should be always selected in order to develop comparable methodologies. In this section, some QA/QC measures are proposed and discussed such as the nature of the solvents (i.e. water, methanol and acetonitrile) for the appropriate elution of compounds of interest and satisfactory peak shapes into the chromatographic system. The same rule must be applied for the selection of modifiers of mobile phase composition used to improve the ionization of some compounds. A set of procedural blanks, solvents blanks and mix of suitable standards will be analyzed in each chromatographic run to study peak characteristics, carry over, contamination or other issues.

3.3. Mass spectrometry (MS)

Considering the large number of possible technical and parameterization approaches (data dependent or independent acquisition, ionization mode, etc.), it is difficult to establish a detailed list of QA/QC measures. Most important of them are: 1) daily calibration 2) defined of minimal value for the signal to noise, 3) prevention of detector saturation and 4) coherence between scan speed and number of cycles.

3.4. Compound identification

This is the most subjective stage of the workflow, because most steps are defined by the experience of the analyst and the available software (vendor and open access). Thus, the establishment of QA/QC measures for this step is essential in order to obtain comparable results. For this purpose, the use of Schymanski-scale to clarify the level of identification recahed for each compound is strongly recommended [5]. In addition. the utilisation of different libraries for compound identification and benchmarking studies can offer a real solution.

3.5. Results

Although the CECs present in human urine are (yet) unknown, a good point to evaluate the whole non-target/suspect screening workflow may be the comparison with the obtained results by applying available well-defined target approaches to the same urine samples.

4. Conclusions

The establishment of a detailed list of QA/QC measures represents a good starting point for the harmonisation of non-target/suspect screening methodologies used in human urine analysis. However, more effort in this direction is still needed owing to the premature status of the major workflows in this field.

5. References

[1] Sauvé S, Desrosiers M. 2014. A review of what is an emerging contaminant. Chem Cent J. 8(15).

[2] Prichard E, Barwick V, 2007. Quality Assurance in Analytical Chemistry. DOI:10.1002/9780470517772

[3] Oberacher, H.; Arnhard, K. 2016. Current status of non-targeted liquid chromatography-tandem mass spectrometry in forensic toxicology. Trends in Anal Chem 84: 94-105.

[4] Oberacher, H. A., K. 2015. Compound identification in forensic toxicological analysis with untargeted LC-MS-based techniques. Bioanalysis 7(21):2825-2840.

[5] *Schymansk EL.;, Jeon J.; Gulde R.; Fenner K., Ruff† M.; Singer† H.P.; Hollender J. 2014. Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. Environ. Sci. Technol., 48 (4): 2097–2098*

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