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Development of the First Metabolite-based LC-MS^n Urine Drug Screening Procedure

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1. Introduction

Screening for toxic compounds or drugs (TCD) in different body samples is one of the major tasks in clinical and forensic toxicology as well as in doping control. Several procedures using different separation and/or detection systems were used for screening purposes in the field of analytical toxicology [1]. While immunoassays allow a quick screening for a limited number of targets, chromatographic methods like photodiode array detector coupled to liquid chromatography (LC) allows a broad screening for TCD. However, mass spectrometry (MS) is widely used in bioanalytics as this technique provides higher sensitivity and identification power in comparison to other methods. Different hyphenations of MS and their current use in analytical toxicology are reviewed elsewhere [2-8] but will be shortly discussed as follows:

Hyphenation of gas chromatography to MS (GC-MS) revolutionized the field of analytical toxicology as this robust and rather cheap technique allows detecting TCD in low concentrations. Therefore, screening procedures and comprehensive reference libraries using electron ionization (EI) spectra had been developed in addition to sophisticated search algorithms [3, 4, 6, 9-14]. GC-MS screening methods became "gold standard" in analytical toxicology according to concentration- and instrument-independent EI spectra and excellent screening results [2, 3, 6]. However, GC-MS is limited to volatile and more or less apolar compounds.

Hyphenation of liquid chromatography to MS (LC-MS) provides higher sensitivity for most of the TCD and overcomes the limitations of GC-MS. By introduction of tandem LC-MS techniques (LC-MS/MS) such as ion trap technology, concentration-independent and reproducible collision-induced dissociation (CID) LC-MS/MS spectra could be obtained which is a prerequisite for comprehensive reference libraries. However, these CID spectra are still restricted to a certain instrument type of a specific manufacturer [15]. Therefore, several LC-MS screening approaches using different instrumentation types have been developed [3, 5, 7, 8, 16-21, 21-33].

In contrast to established GC-MS libraries containing parent compound and metabolite spectra [9-11], current commercially available LC-MS/MS libraries [9, 23, 30] were lacking of metabolite reference spectra. This limits their applicability for urine screening.

However urine is still the best sample for comprehensive screening approaches, as most of the TCDs are excreted more or less metabolized in high concentrations in urine [34]. Detection of various metabolites increases the selectivity and lowers the detection limits of a compound. Accordingly, there is a decreased risk of false negative screening results. In addition, the risk of false positives is also limited as the detection of metabolites confirms the body passage and thereby the intake of a particular TCD.

2. Aims and Scopes

The aim of this work was to develop the first metabolite-based LC-MS screening procedure which provides comprehensive urine screening results and therefore complements the current gold standard GC-MS approach [10, 11].

With the regard to detect TCD and the corresponding metabolites in urine, the scopes of this work were firstly to develop an MS method, secondly a chromatographic system and thirdly a universal sample preparation. To achieve comprehensive screening results, a reference library containing thousands of CID spectra including parent compounds as well as metabolites had to be build up in addition to automatic data evaluation systems.

As LC-MS/MS CID spectra are still restricted to a certain instrument type of a specific manufacturer, the instrument transferability of the developed LC-MS screening had to be investigated additionally.

3. Results and Discussion

In order to provide comprehensive urine screening results, a linear ion trap-based data dependent acquisition (DDA) MS^n method was used. MS^2 and MS^3 spectra were collected after a survey MS^1 full scan using electrospray ionization. The developed chromatographic system using a 1.9 µm C18 analytical column provided high separation performance. Sample preparation was conducted by precipitation of 100 µL urine using 400 µL acetonitrile, centrifugation, and evaporation of the supernatant. After that the residue was resolved in 50 µL of a mixture of mobile phase I and II and analyzed by the LC-MS system [35, 36].

In Figure 1 the general workflow for the LC-MS^n screening is depicted.

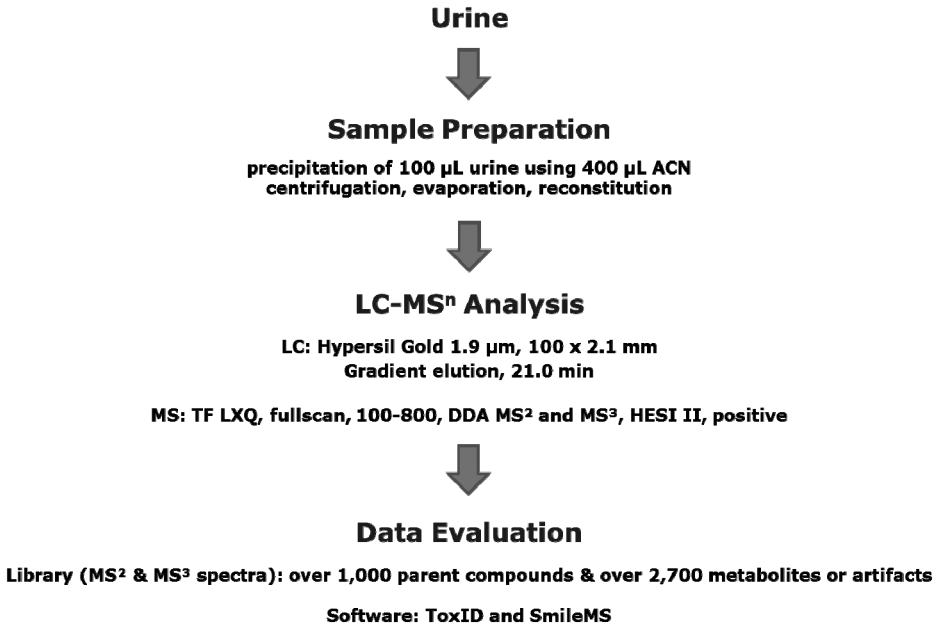

Fig. 1. General workflow of the LC-MS^n screening

Mass spectra of the parent compounds were recorded from methanolic stock solutions (1 mg/L) and those of the metabolites in rat or human urine after workup and LC separation. They were stored in the library using the NIST (National Institute of Standards and Technology, Gaithersburg, MD) library format by the NIST mass spectral search program.

The library consists of MS^2 and MS^3 spectra of over 1,000 toxicologically relevant parent compounds and over 2,700 metabolites or artifacts [35, 36]. In comparison to other LC-MS libraries [19, 21, 23, 30, 32, 33], this reference library is the most comprehensive in view of metabolites or artifacts of TCD. In addition to that, about 100 endogenous biomolecules and impurities, and about 50 unknown compounds containing common structure elements of compounds are stored in the library.

Excellent screening results could be obtained for the analysis of authentic urine, as shown by a comparison study. In this study, 150 urine samples were screened by the new LC-MS screening as well as by the well established GC-MS approach. Overall, both screening methods provide similar screening results. Nevertheless, it must be mentioned that both systems are complementary to each other. On the one hand, LC-MS was able to better detect more polar cardiovascular drugs - a known gap of the GC-MS screening approach - on the other hand, GC-MS was able to detect certain benzodiazepines via benzophenones more sensitively. This extraordinary high sensitivity is achieved according to the sample preparation step which is used for the GC-MS analysis.

However the developed LC-MS screening approach is nowadays a fundamental part of the systematic toxicological analysis (STA) in the lab of Prof. Dr. Dr. h.c. Hans H. Maurer and showed good robustness in the analysis of thousands of authentic samples.

In addition, it was shown that the developed screening concept and reference library can be transferred to a QTrap system. Because of the huge amount of metabolite reference spectra, it was possible to detect approx. 90 % of the drugs by the QTrap system in comparison to the LXQ reference system by analyzing 100 authentic urine samples on both LC-MS systems [37].

In conclusion, the presented work provided a unique screening concept including the most comprehensive metabolite-based LC-MS reference library in addition to systematic data evaluation of the screening results for LXQ and QTrap LC-MS systems. The developed screening is a fundamental part of STA as this technique is closing and minimizing analytical gaps provided by gold standard GC-MS screening methods. According to this, further systematic implementation and investigations will be performed to further improve the screening results and the LC-MS platform independent use.

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