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Online extraction LC-MS^n method for the detection of drugs in urine, serum and heparinized plasma

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Abstract

Aim: In clinical toxicology, fast and specific methods are necessary for the screening of different classes of drugs. Therefore, an online extraction LC-MS^n method using a MS^2 and MS^3 spectral library for the identification of toxicologically relevant xenobiotic substances has been developed and validated.

Methods: Urine samples were run twice, once native and once after enzymatic hydrolysis. Serum and heparinized samples were run once only. Internal standards as well as buffer or acetonitrile were added to urine or serum and heparinized plasma, respectively. Following centrifugation, the supernatant was injected into the system. Extraction was performed by online turbulent flow chromatography. Chromatographic separation was achieved using a phenyl/hexyl column. For detection, a linear ion trap, equipped with an APCI interface, was used and the different compounds were identified using a MS^2 and MS^3 spectral library containing more than 450 compounds.

Results: The turn-around time to report the results was less than 1 hour for serum and heparinized plasma samples and approximately 2 hours for urine samples including hydrolysis. About 90 % of the over 450 substances could be identified with a limit of identification below 100 ng/ml in all sample materials. The recovery was > 90 % for 97 % of the tested substances, and there was no matrix effect for 89 % of the tested substances. Carryover could be well controlled and the method had a good reproducibility (coefficients of variation < 2.5% for the retention times, < 0.07 % for the mass-to-charge ratio and < 7.2 % for the spectral reproducibility). A patient sample comparison with existing methods for urine as well as a comparison between screening results in urine and serum or heparinized plasma and urine gave satisfactory results.

Conclusions: The presented method allows a fast and sensitive analysis of a broad range of compounds in different matrices.

1. Introduction

As complementary method for immunological drug of abuse screening methods and GC-MS screening, LC-MS screening becomes more and more popular. Until today, GC-MS is regarded as gold standard in the field of clinical toxicological screening. However, all substances potentially involved in intoxications cannot be analyzed using GC-MS. For example, the analysis of thermolabile substances and compounds with a high molecular weight is not possible using this technique. Moreover, GC-MS needs extensive, mostly manual sample preparation that slows down the process and can be an origin of errors.

Here, we present a fast and sensitive screening method using online turbulent flow chromatography for the sample preparation, thereby omitting extensive manual sample preparation steps.

2. Material and Methods

2.1. Sample Pre-Treatment

Urine samples were run twice, once natively and once after enzymatic hydrolysis using beta-glucuronidase/arylsulfatase from *Helix pomatia*. After dilution with ammonium acetate buffer and addition of a mixture of three internal standards (temazepam-d5, haloperidol-d4 and morphine-d3), samples were centrifuged and injected into the LC-MS system.

After addition of the same mixture of internal standards as for urine, serum and heparinized plasma samples were precipitated with acetonitrile. Following centrifugation, samples were injected into the LC-MS system.

2.2. LC-MS system

The HPLC system consisted of a Transcend TLX-1 HTLC, equipped with two Allegro pumps, an HTC PAL autosampler and a valve interface module with built-in switching valves, all controlled by Aria software (version 1.6.2). For extraction, a combination of two columns (Cyclone and C18 XL, both Thermo Fisher Scientific, Basel, Switzerland) was used. Extraction was performed under alkaline conditions. For the analytical chromatography, a Betasil phenyl/hexyl column (Thermo Fisher Scientific) with eluents consisting of ammonium acetate, water, methanol and formic acid was used. The gradient is described in [1].

The MS analysis was performed using a LXQ linear ion trap mass spectrometer, controlled by XCalibur 2.0.7 SP1 software (all Thermo Fisher Scientific, Basel, Switzerland). As interface, an atmospheric pressure chemical ionization (APCI) source was used. Acquisition was performed in data-dependent acquisition mode. Both, MS^2 and MS^3 spectra were recorded, and polarity was switched constantly between positive and negative mode. A spectral library was built in-house by direct infusion of substances to the MS. For the automated processing of chromatograms, ToxID 2.1.1 (Thermo Fisher Scientific, Basel, Switzerland) was used.

2.3. Method Validation

Aliquots of pooled blank matrices (urine, serum and heparinized plasma) from different healthy volunteers not taking any medications were spiked with mixtures of different substances to achieve a concentration of 10, 100, 1000 and 10’000 ng/ml of each substance. The lowest concentration where a substance could be identified by ToxID was considered as the limit of identification for the corresponding matrix.

Recovery and matrix effects were checked in a method in analogy to Matuszewski et al. [2] in a subset of 47 representative compounds. Recovery was calculated by dividing the peak area of the neat standards injected onto the extraction columns with subsequent analytical chromatography by the peak area of neat standards injected directly onto the analytical column, omitting the online extraction step. The matrix effect was calculated by dividing the peak area of a spiked matrix sample (urine, serum and heparinized plasma) by the peak area of a standard in solvent. Carryover was determined by injection of urines spiked at high concentrations (10’000 ng/ml). Reproducibility of the retention times, the mass-to-charge ratio and the spectral reproducibility was checked both within and between days.

2.4. Patient Samples

A total of 103 patient urine samples which were already analyzed by the GC-MS procedure [3], existing LC-MS^2 screening methods [4] and immunological screening methods were taken out of the archive of the routine laboratory and re-analyzed with the new online extraction LC-MS^n screening method.

To check the performance of the screening system for heparinized plasma (which is usually available in the clinical-chemical laboratory for the determination of emergency parameters), a total of 47 samples from patients were analyzed from whom urine and heparinized plasma samples drawn on a similar time point were available.

3. Results and Discussion

3.1. Method Validation

The limits of identification are displayed separately for urine, serum and heparinized plasma in Fig. 1. About 90 % of the over 450 substances could be identified with a limit of identification below 100 ng/ml in all sample materials.

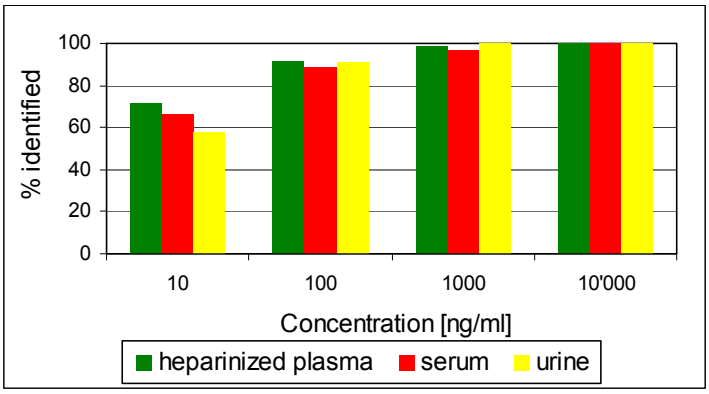

Fig. 1. Limits of identification for heparinized plasma, serum and urine.

Matrix effect and recovery data are shown in Fig. 2. The maximal ion suppression was 35 % in urine for one of the tested substances. 86 % of the tested substances did not show ion suppression. In serum, maximum ion suppression was 15 %, and 90 % of all substances did not show any ion suppression. No ion suppression was observed in heparinized plasma. The recovery was > 90 % for 97 % of the tested substances, which demonstrates the ability of the online extraction system to extract substances out of a wide polarity range.

The carryover for all substances was below 1% for all substances in the first blank sample after injection of the highly concentrated spiked urine. In the second blank, no substances have been identified in all cases. To exclude the risk for carryover, a blank was run after every patient sample.

The method had a good reproducibility; coefficients of variation were below 2.5% for the retention times, below 0.07 % for the mass-to-charge ratio and below 7.2 % for the spectral reproducibility.

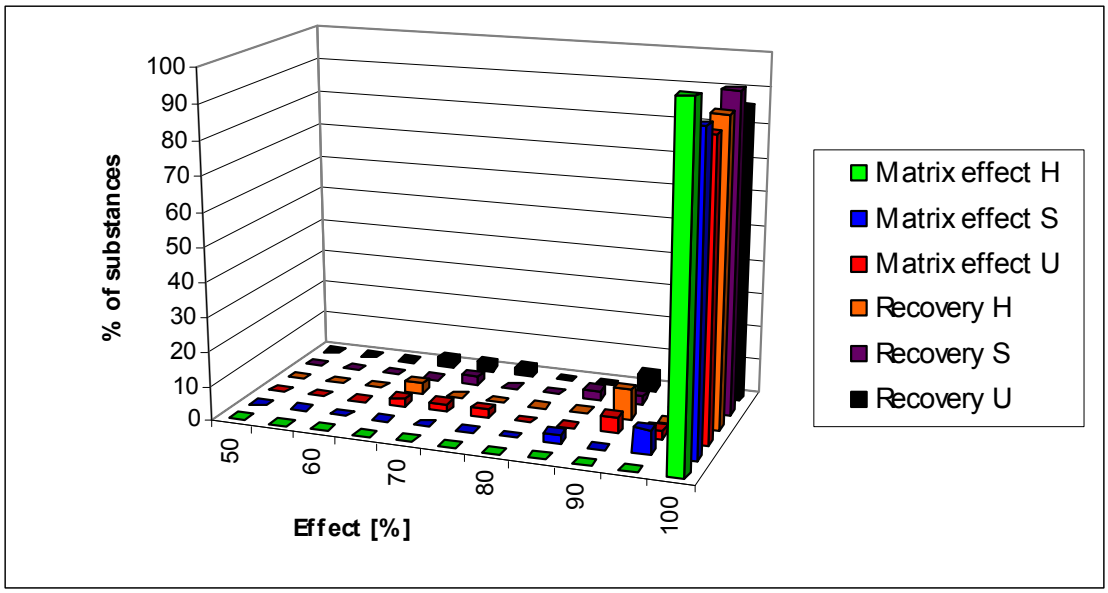

Fig. 2. Matrix effect and recovery data for heparinized plasma (H), serum (S) and urine (U).

3.2. Patient Samples

Among 103 patient urine samples, a total of 451 substances could be identified using the combination of both, the established methods and the new method (Fig. 3). When using only the established methods, 354 substances (78 %, 106 different compounds) could be found. Applying only the method presented, 404 substances (89 %, 100 different compounds) could be identified. For 7 patient samples, no compounds have been detected. The agreement between the established method and the new method is good.

The results between urine and heparinized plasma matched well. 20 out of 47 cases (43 %) gave completely identical results in both matrices. On a substance level, the agreement between urine and heparinized plasma was in average 71 %, taking into account all cases.

Differences may to a large extent be explained by the different time points of withdrawal of the urine and plasma samples as well as by the longer detection windows in urine. Plasma samples are the better choice regarding the monitoring of the current drug exposure of a patient, whereas urine is superior in the retrospective evaluation of the drugs taken by the patient.

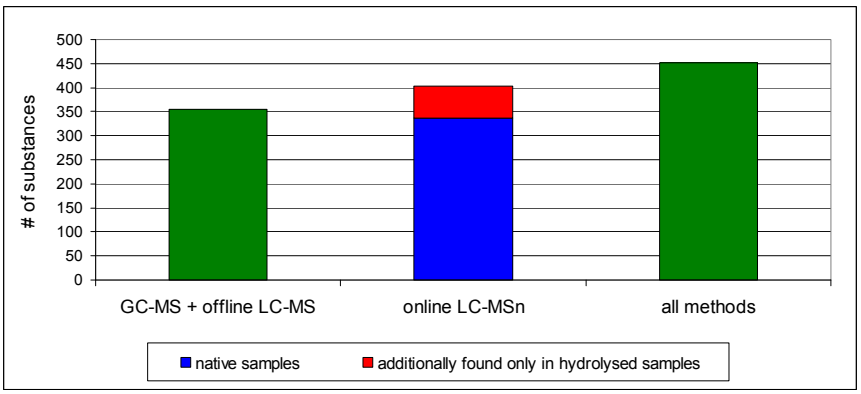

Fig. 3. Patient sample comparison for the urine samples.

4. Conclusion

The presented method allows a fast and sensitive analysis of a broad range of compounds in urine as well as in serum or heparinized plasma. The patient sample comparisons demonstrated the suitability of the method for routine usage on a daily basis.

5. References

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