Comprehensive systematic toxicological analysis of human body fluids by parallel LC/MS/MS and GC/MS analysis

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Abstract

Aim: GC/MS is a well established method in our laboratory for systematic toxicological analysis (STA) of human body fluids. Our goal was to develop an untargeted LC/MS/MS assay to complement the existing GC/MS screening assay.

Methods: The LC/MS/MS approach developed for untargeted screening analysis used MS/MS under data-dependent acquisition control (DDA) to identify compounds by subsequent library search. Samples were processed by a generic solid-phase extraction method. The eluate was split for parallel GC/MS and LC/MS/MS analysis.

Results: By analysing blank samples, spiked samples, certified reference materials, proficiency test samples and authentic casework samples, the performance of the developed LC/MS/MS screening procedure was characterized and compared to GC/MS screening. The experimental results clearly proved that the additional use of LC/MS/MS analysis increased the reliability of STA results.

Conclusion: The developed procedure employing parallel GC/MS and LC/MS/MS analysis of processed human body fluids represents a competent approach for comprehensive STA and was successfully integrated in our laboratory services.

1. Introduction

Systematic toxicological analysis (STA) is a major part of the examination in forensic and clinical toxicology. STA is aimed at detecting and identifying all substances of toxicological relevance (i.e. drugs, drugs of abuse, poisons and/or their metabolites) in biological material. Particularly, gas chromatography–mass spectrometry (GC/MS) is a routinely applied screening and confirmation tool in STA [1]. There is clear trend, however, to complement existing GC/MS procedures with liquid chromatography-mass spectrometry (LC/MS) assays. One competent LC/MS-based approach for untargeted screening analysis makes use of MS/MS under data-dependent acquisition control (DDA) to identify compounds by subsequent library search [2,3].

2. Material and Methods

The solid-phase extraction (SPE) procedure existing for GC/MS-based STA was adapted for parallel GC/MS and LC/MS/MS analysis. SPE was performed on Spe-ed Scan ABN columns (200 mg/3 mL, Applied Separations). The eluate was split for parallel GC/MS and LC/MS/MS analysis.

LC was performed on a Eurosphere C18 column (100 × 2 mm, 5 µm, Knauer). A QTRAP 3200 system (AB Sciex) was used for ESI-MS/MS in positive ion mode under data-dependent

acquisition control. The obtained MS/MS spectra were matched to the "Wiley Registry of Tandem Mass Spectral Data, MSforID" (Wiley).

The GC-MS system consisted of a HP7890 GC device with a HP5975C inert XL mass-selective detector. A DB-XLB column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, J&W) was used for chromatographic separations. The obtained spectra were matched to the Maurer-Pfleger-Weber mass spectral library (Wiley).

3. Results and Discussion

GC/MS is a well established method in our laboratory for STA of human body fluids. The developed LC/MS/MS assay was aimed to complement the existing GC/MS assay. Parallel screening should help to increase the reliability of STA by increasing the range of detectable compounds and by confirming the presence or absence of compounds by two independent methods. Our goal in method development was to change as much as is necessary and as little as is possible of the existing GC/MS screening workflow. Thus, samples suitable for both screening methods were obtained by splitting the eluate after SPE.

By analysing blank samples, spiked samples, certified reference materials, proficiency test samples and authentic casework samples, the performance of the developed LC/MS/MS screening procedure was characterized. Parameters determined included selectivity, detection sensitivity and reliability of identification.

Blank samples were analyzed to evaluate the selectivity of the LC/MS/MS method (Figure 1). Typically, <5% false positive matches were obtained by automated library search. In all cases, however, identity was excluded by visual inspection of the corresponding spectra. A large number of correct negative results were obtained indicating the need for adding spectra of endogenous compounds to the library.

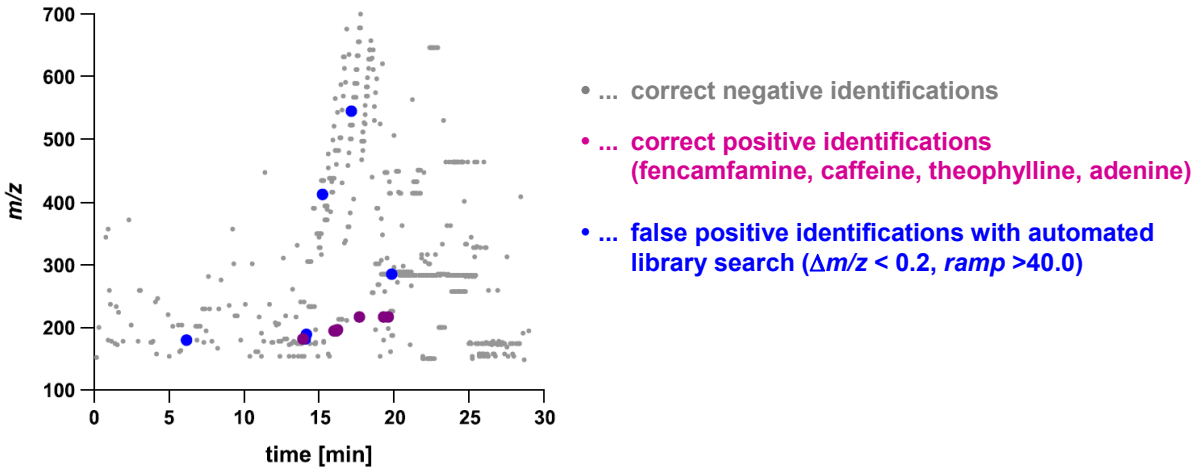

Fig. 1. Results for screening a blank plasma sample with the developed LC/MS/MS assay.

The detection sensitivity of the LC/MS/MS assay was evaluated by adding defined amounts of reference compounds to blank plasma samples. For basic compounds with a plasma concentration >20 ng/ml hardly any false negative results were obtained. Due to the use of ESI in positive ion mode, however, acidic compounds were less efficiently detected. The situation might be improved by additional scanning in the negative ion mode.

The screening procedure combining GC/MS and untargeted LC/MS/MS analysis was recently implemented in our laboratory services and has been successfully applied for the analysis of >250 authentic casework samples. Due to the fact that the majority of compounds were detected by two independent methods, the reliability of STA results was improved (Figure 2). Furthermore, by parallel screening the number of detected compounds was significantly increased. Despite considerable success of compound identification with the Wiley Registry MS/MS, however, a further improvement of the success rate seems to be achievable by adding more reference spectra; particularly spectra representing abundant drug metabolites should be added.

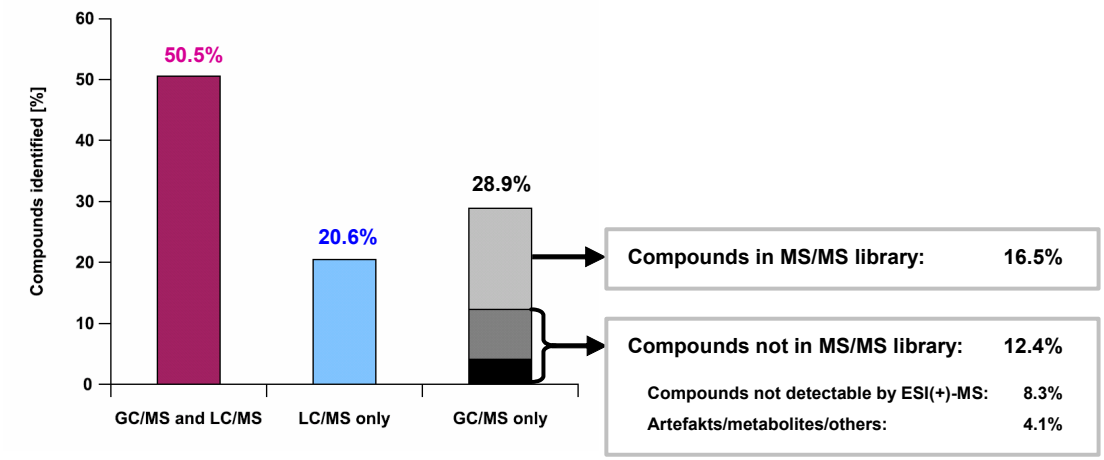

Fig. 2. Comparison of GC/MS and LC/MS/MS results obtained by screening 164 serum/blood samples. Overall, 194 compounds were identified.

4. Conclusions

We have developed a procedure for comprehensive STA employing parallel GC/MS and LC/MS/MS analysis of processed human body fluids that was successfully integrated in our laboratory services.

5. References

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[2] Peters FT. Recent advances of liquid chromatography-(tandem) mass spectrometry in clinical and forensic toxicology. Clin Biochem 2011;44:54–65.

[3] Oberacher H, Schubert B, Libiseller K, Schweissgut A. Detection and identification of drugs and toxicants in human body fluids by liquid chromatography-tandem mass spectrometry under data-dependent acquisition control and automated database search. Anal Chim Acta 2013;770:121-131.