Hyphenated high-resolution mass spectrometry—the “all-in-one” device in analytical toxicology?

Hans H. Maurer

Received: 1 October 2020 / Revised: 29 October 2020 / Accepted: 11 November 2020 / Published online: 28 November 2020
© The Author(s) 2020

Abstract
This trend article reviews papers with hyphenated high-resolution mass spectrometry (HRMS) approaches applied in analytical toxicology, particularly in clinical and forensic toxicology published since 2016 and referenced in PubMed. The article focuses on the question of whether HRMS has or will become the all-in-one device in these fields as supposed by the increasing number of HRMS presentations at scientific meetings, corresponding original papers, and review articles. Typical examples for the different application fields are discussed such as targeted or untargeted drug screening, quantification, drug metabolism studies, and metabolomics approaches. Considering the reviewed papers, HRMS is currently the only technique that fulfills the criteria of an all-in-one device for the various applications needed in analytical toxicology.

Keywords High-resolution • Mass spectrometry • All-in-one device • Screening • Quantification • Metabolism • Metabolomics • Toxicology

Introduction
Since the 1980s, gas chromatography-mass spectrometry (GC-MS) has become the gold standard in analytical toxicology with selected ion monitoring (SIM) for immunoassay confirmation, targeted screening, and quantification. Full-scan monitoring providing informative and reproducible mass spectra with electron impact (EI) ionization allows comprehensive screening with a high degree of confidence using corresponding reference libraries [1–6]. In last years, the number of GC-MS papers decreased, but GC-MS with electron ionization (EI) is still in use as the backbone of the clinical and forensic laboratory [3].

Since the 1990s, liquid chromatography-mass spectrometry (LC-MS) with electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), or atmospheric pressure photoionization (APPI) revolutionized the bioanalysis also in analytical toxicology. LC coupled to tandem MS (LC-MS/MS) with selected reaction monitoring (SRM) for targeted (multi-analyte) screening and quantification or with data-dependent or data-independent product ion spectra formation for comprehensive screening has become a new gold standard [2–6].

The next trend started in the last years with the coupling of high-resolution mass spectrometry (HRMS) mostly with GC or LC for analysis of small and large molecules in analytical toxicology [2]. HRMS was developed in the 1960s with double-focusing mass spectrometers, but today time-of-flight (TOF) or Orbitrap (OT) mass analyzers are common, mostly as hybrids with triple quadrupoles (QTOF, QOT) or ion traps in front allowing fragmentation to reproducible MS/MS spectra [3–7]. The high mass resolution allows differentiation of isobaric compounds with the same nominal mass but different elemental compositions. Thus, mass traces of coeluting isobaric compounds, e.g., endogenous biomolecules, can be excluded increasing the selectivity and thus sensitivity. The elemental composition of a molecule can be calculated by accurate determination of the parent and fragment masses allowing provisional identification of unknown compounds, e.g., by comparing with lists of the exact masses and empirical formulas of potential poisons [8]. However, isomeric compounds can only be differentiated by different fragmentation [9]. The increasing number of HRMS presentations at scientific meetings, corresponding original papers, and review articles...
[2–7, 10–12] may lead to the presumption that HRMS has or will become the all-in-one device for targeted or non-targeted (also named untargeted) screening, quantification, drug metabolism, and metabolomics in analytical toxicology, namely in clinical and forensic toxicology, forensic chemistry, doping control, etc. Therefore, the aim of the present trend article is to confirm this presumption considering English-written papers published since 2016 and referenced in PubMed preselected with the terms “HRMS and (toxicology or forensics or doping).” Out of them, review articles covering a particular aspect of a trend and typical—exemplary—original papers supporting the corresponding trends have been selected as the number of the citations is limited for trend articles.

**Screening for detection of drugs, poisons, and/or their metabolites**

In contrast to other fields of analytical chemistry, the analysis in clinical and forensic toxicology has to start with a screening for detection of often unknown drugs or poisons. Depending on the case and the clinical signs of intoxication, the screening has to cover a limited number of compounds or even over 10,000 potential poisons. Thus, the analytical strategy is different; either a targeted screening or a non-targeted comprehensive screening can be performed.

LC-MS/MS in the SRM mode was established over the last years as the standard for multi-analyte targeted screening, often combined with quantification [2, 13–15]. The identification power depends, of course, on the selectivity and the number of monitored transitions. Selectivity can markedly be improved by using HRMS doubling the identification points per selected ion [16]. Another advantage of HRMS is the option of combined targeted and untargeted screening. Thoren et al. [17] compared a typical LC-MS/MS targeted screening with a triple quadrupole linear ion trap with a non-targeted LC-HRMS/MS method with advantages for general unknown drug screening. Both methods used information-dependent acquisition of product ion spectra. LC-HRMS/MS was slightly less sensitive, but offered an open unknown screening. Further advantages will be discussed in the following.

Interestingly, GC coupled to HRMS (GC-HRMS) was applied for a high-throughput screening for detection of about 300 drugs and poisons in human blood using an OT analyzer [18]. However, considering the limitations of GC [9] such as risk of thermal degradation, limited volatility without derivatization, and less sensitivity, the advantage over corresponding LC-HRMS approaches cannot be assessed. In the last few years, a clear trend to highly selective and sensitive screening by LC-HRMS/MS with QTOF or QOT analyzers was observed [19–21], particularly since hundreds of so-called new psychoactive substances (NPS) appeared on the drugs of abuse market per year [2–6, 12, 22]. Pasin et al. [11] critically reviewed applications for NPS analysis and highlighted the advantage to detect and tentatively identify novel analogs without the need for certified reference materials or comprehensive mass spectral libraries. They discussed non-targeted screening strategies as a two-step process that involves the discovery or detection of a component followed by putative identification. Component discovery has been identified as the most problematic step, which can be categorized into two different approaches, top-down or bottom-up, as illustrated in Fig. 1. The current role of HRMS in NPS analysis was recently discussed with experts in this field [23]. Considering all advantages, HRMS tend to replace conventional quadrupole-based MS, particularly using integrated targeted/non-targeted screening for detection of known and new substances also with retrospective data mining [24].

In the following, selected examples for typical LC-HRMS screening approaches are discussed showing the trend of universality of this technique. In direction to compliment or even replace well-established GC-MS general unknown screenings [25–27], Helfer et al. [20] developed an LC-QOT-MS/MS standard urine screening approach in full-scan mode after positive/negative switching and data-dependent acquisition for unknowns. A compound was positively identified when the corresponding accurate mass precursor ion and the five most intense fragment ions were detected and the MS/MS spectrum fits well with the corresponding full HR-MS/MS reference library of parent drugs and their metabolites [28]. This approach was successfully transferred to blood analysis providing fast, simple, and robust screening and identification of a broad range of drugs within therapeutic ranges [21]. However, in contrast to GC-EI-MS reference libraries (e.g., ref. [29]) running with different apparatus types, LC-(HR)MS libraries (e.g., ref. [28]) can be more apparatus depending as ionization, collision energy, MS/MS conditions, etc. may have a significant influence on the transferability, but can be limited by adopting and standardizing these parameters [30]. Partridge et al. [31] described another comprehensive LC-QTOF-MS/MS blood screening also using data-dependent acquisition, and an in-house retention time, accurate mass, and MS/MS spectral database. As advantage of such methods, they can be easily updated with new compounds without affecting method performance. Finally, an LC-QTOF-MS/MS with data-independent acquisition was developed for serum screening and applied to authentic serum and post-mortem femoral blood samples in comparison to GC-MS [32]. Not surprisingly, the HRMS method could detect much more drugs than the GC-MS approach.

Besides these general approaches, various methods were published for particular drug groups e.g., hallucinogenic phenethylamines (non-targeted) [33], low-dosed opioids (non-targeted data acquisition coupled with targeted data processing) [34], or synthetic cannabinoids (non-targeted) [35]. Thanks to its high sensitivity, LC-HRMS/MS was successfully applied also for broad-spectrum drug screening in
low sample volumes such as dried blood spots [36] or in samples with low concentrations such as hair samples [37], urine after dilute-and-shoot application [38], or wastewater [39]. Finally, Mollerup et al. [40] described a new approach with LC-ion mobility-HRMS/MS for broad scope screening based on prediction of collision cross section and retention time with machine learning using artificial neural networks. Together with the exact mass, tentative identification of new compounds could be performed with in silico predicted reference values for improving confidence and filtering false-positive identifications.

Besides GC or LC coupling, paper spray ionization coupled to QOT (PSI-HR-MS/MS) allowed comprehensive urine drug screening [41]. Its screening power was compared to that of published LC-HR-MS/MS procedures [42] showing that PSI-HR-MS/MS was suitable, but limitations should be considered such as limited detection of drugs in low concentrations and risk of false-positive or false-negative results caused by mixed spectra. McKenna et al. [43] compared PSI-HR-MS/MS with conventional LC-MS/MS resulting in acceptable qualitative and quantitative agreement. A further ambient coupling was described by Duvivier et al. [44] using direct analysis in real-time HRMS for drug testing by longitudinal scan of intact locks of hair. Data-dependent product ion scanning allowed detection of various drugs of abuse in a single hair confirmed by accurate mass and fragmentation patterns. In forensic chemistry, drugs (e.g., NPS) in solid and liquid samples could be detected using ambient ionization.
techniques coupled to HRMS [45], namely by laser diode thermal desorption or atmospheric solids analysis probe allowing fast analysis of a wide range of samples with minimal or no sample preparation. This ambient coupling confirmed again the universality of HRMS.

Quantification

For assessing the extent of impairment or severity of poisoning, quantification mainly in blood (plasma, serum) is needed. So far, LC-MS/MS mostly in SRM mode is the method of choice for quantification, often combined with targeted screening (see above), allowing multi-analyte approaches saving time and resources [2, 4, 6, 13, 46]. The question arises whether there is a trend that HRMS will take over also this field. Recent papers and review articles clearly indicate this, particularly for low-dosed drug or in low-volume samples [7, 11, 22]. For example, Caspar et al. [47] developed a quantitative approach for low-dosed hallucinogens and opioids in blood plasma using LC-OT-MS/MS with alternating HR full-scan and all-ions fragmentation MS. This allowed identification and quantification with no limitations on the number of monitored compounds and reevaluation of the acquired data using group-indicating fragment ions, e.g., for new or unexpected analytes. Thomas et al. [48] described simultaneous quantification of insulin, its synthetic analogs, and C-peptide in human plasma by LC-OT-MS/MS with targeted single ion monitoring experiments for the multiply protonated precursors of the target peptides or alternatively with product ion experiments for the respective five- or fourfold protonated precursors. Further procedure for insulins was recently reviewed by the same group [49] concluding that HRMS provides the sensitivity required to determine analyte concentrations in the sub-ng/mL level. Another highly sensitive approach was published [50] for determination of anticoagulant rodenticides in blood by LC-QTOF-MS/MS with parallel reaction monitoring providing the highest sensitivity. Finally, Kronstrand et al. [51] developed an LC-QTOF-MS/MS method using the all-ions mode for quantification of low concentrations of drugs in hair showing that HRMS found its way also in alternative matrix testing.

Metabolism of drugs of abuse

Studies on drug metabolism are mandatory in drug discovery and development and toxicological risk assessment, and also for developing urine screening assays particularly for lipophilic drugs detectable often only as metabolites in urine [7]. For example, NPS are sold without any preclinical study, and thus, no or limited information about their excretion form is known. Thus, clinical and forensic toxicologists started with analytical strategies for identification of the metabolites and their formation pathways [7, 52–55] using animals or human in vivo, ex vivo, or in vitro samples such as blood, urine, primary hepatocytes, cell cultures, S9 fraction, microsomes, or cytosol. Various review articles [7, 52, 54–57] confirm that HRMS plays the major role in this field, particularly in non-targeted modes allowing retrospective data mining [56]. Again, HRMS provides the elemental composition of the parent and fragment masses allowing to identify the type of metabolic changes and in most cases the position in a particular part of the molecule, but not the exact position, e.g., in an aromatic ring system [7]. However, the latter is of minor relevance in developing urine screening assays.

Metabolomics techniques in analytical toxicology

Since the last few years, metabolomics plays also a role in clinical and forensic toxicology and doping control. Besides conventional GC- or LC-MS methods, LC-HRMS was established particularly for untargeted metabolomics studies, again because of its high specificity, sensitivity, and flexibility [58–63]. There are two main application fields, one focusing on the change of the endogenous compounds under the influence of drug administration [62, 64–69] or sample manipulation [65, 70–74] and one on the use of metabolomics techniques for investigating the metabolism of new drugs, namely of NPS [60, 61, 75]. Metabolomics could also play a role in doping control, e.g., for detecting hormone abuse considering that hormones have a strong influence on human endogenous metabolism changing several endogenous parameters [76].

Outlook

The papers reviewed in this article clearly show that HRMS is currently the most powerful and flexible technique in analytical toxicology used for various applications such as targeted and non-targeted screening, quantification, drug metabolism, and metabolomics. Of course, also for HRMS, potential pitfalls have to be considered and details can be found in ref. [9]. Today, HRMS is the only technique that fulfills the criteria of an all-in-one device for the various applications needed in analytical toxicology. It can be expected that HRMS will become the gold standard and that its application will replace most of the assays with other techniques in future, of course considering suitable separation and/or ionization techniques such as GC with EI or LC with ESI, APCI, or APPI. Current limitations of HRMS techniques are the comparably expensive apparatus and the need of well-skilled operators. Another problem is the enormous size of (full scan) data requiring huge
storage and fast and sophisticated software for data evaluation. Although over time the costs are becoming lower and the software packages have improved, the costs still limit the widespread distribution in routine laboratories and the software needs to become more user-friendly.

Acknowledgments The author would like to thank Prof. Dr. Markus Meyer for reviewing the manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL.

Compliance with ethical standards

The content of this article requires no approval from an ethics committee.

Conflict of interest The author declares that he has no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Maurer HH. Position of chromatographic techniques in screening for detection of drugs or poisons in clinical and forensic toxicology and/or doping control. Clin Chem Lab Med. 2004;42:1310–24.
2. Maurer HH. Mass spectrometry for research and application in therapeutic drug monitoring or clinical and forensic toxicology. Ther Drug Monit. 2018;40:389–93.
3. Fagiola M. Current and future directions of high resolution and tandem mass spectrometry in postmortem and human performance toxicology. Leg Med (Tokyo). 2019;37:86–94.
4. Brown HM, McDaniel TJ, Fedick PW, Mulligan CC. The current role of mass spectrometry in forensics and future prospects. Anal Methods. 2020;12:3974–97.
5. Liu L, Wheeler SE, Venkataramanan R, Rymer JA, Pizon AF, Lynch MJ, et al. Newly emerging drugs of abuse and their detection methods: an ACLPS critical review. Am J Clin Pathol. 2018;149:105–16.
6. Mogollon NGS, Quiroz-Moreno CD, Prata PS, de Almeida JR, Cevallos AS, Torres-Guerrero R, et al. New advances in toxicological forensic analysis using mass spectrometry techniques. J Anal Methods Chem. 2018;2018:4142527.
7. Maurer HH, Meyer MR. High-resolution mass spectrometry in toxicology: current status and future perspectives. Arch Toxicol. 2016;90:2161–72.
8. Ojanpera S, Pelander A, Pelzing M, Krebs I, Vuori E, Ojanpera I. Isotopic pattern and accurate mass determination in urine drug screening by liquid chromatography/time-of-flight mass spectrometry. Rapid Commun Mass Spectrom. 2006;20:1161–7.
9. Maurer HH. Pitfalls in drug testing by hyphenated low- and high-resolution mass spectrometry. Drug Test Anal. 2020;12:172–9.
10. Kind T, Tsugawa H, Cajka T, Ma Y, Lai Z, Mehta SS, et al. Identification of small molecules using accurate mass MS/MS search. Mass Spectrom Rev. 2018;37:513–32.
11. Pasin D, Cawley A, Bidny S, Fu S. Current applications of high-resolution mass spectrometry for the analysis of new psychoactive substances: a critical review. Anal Bioanal Chem. 2017;409:5821–36.
12. Meyer MR, Maurer HH. Review: LC coupled to low- and high-resolution mass spectrometry for new psychoactive substance screening in biological matrices - where do we stand today? Anal Chim Acta. 2016;927:13–20.
13. Remane D, Wissenbach DK, Peters FT. Recent advances of liquid chromatography-(tandem) mass spectrometry in clinical and forensic toxicology - an update. Clin Biochem. 2016;49:1051–71.
14. Zhang YY, Wei B, Zhu Y, Zhang Y, Bluth MH. Liquid chromatography–tandem mass spectrometry: an emerging technology in the toxicology laboratory. Clin Lab Med. 2016;36:635–61.
15. Seger C, Salzmann L. After another decade: LC-MS/MS became routine in clinical diagnostics. Clin Biochem. 2020;82:2–11.
16. European Union. 2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. https://op.europa.eu/de/publication-detail/-/publication/ed928116-a955-4a84-b10a-cf7a82bad858/language-en. Accessed 20 Sept 2020
17. Thoren KL, Colby JM, Shugarts SB, Wu AH, Lynch KL. Comparison of information-dependent acquisition on a tandem quadrupole TOF vs a triple quadrupole linear ion trap mass spectrometer for broad-spectrum drug screening. Clin Chem. 2016;62:170–8.
18. Pan M, Xiang P, Yu Z, Zhao Y, Yan H. Development of a high-throughput screening analysis for 288 drugs and poisons in human blood using Orbitrap technology with gas chromatography-high resolution accurate mass spectrometry. J Chromatogr A. 2019;1587:209–26.
19. Colby JM, Thoren KL, Lynch KL. Suspect screening using LC-QqTOF is a useful tool for detecting drugs in biological samples. J Anal Toxicol. 2018;42:207–13.
20. Helfer AG, Michely JA, Weber AA, Meyer MR, Maurer HH. LC-HR-MS/MS standard urine screening approach: pros and cons of automated on-line extraction by turbulent flow chromatography versus dilute-and-shoot and comparison with established urine precipitation. J Chromatogr B Anal Technol Biomed Life Sci. 2017;1043:138–49.
21. Helfer AG, Michely JA, Weber AA, Meyer MR, Maurer HH. Liquid chromatography-high resolution-tandem mass spectrometry using Orbitrap technology for comprehensive screening to detect drugs and their metabolites in blood plasma. Anal Chim Acta. 2017;965:83–95.
22. Wagemann L, Maurer HH. Bioanalytical methods for new psychoactive substances. Handb Exp Pharmacol. 2018;252:413–39.
23. Straftmann FG, Lynch KL, Krotulski A, Negri P, Cichelli J, Meyer MR. Challenges of high-resolution mass spectrometry for detecting designer drugs. Clin Chem. 2020;66:868–74.
24. Wu AH, Colby J. High-resolution mass spectrometry for untargeted drug screening. Methods Mol Biol. 2016;1383:153–66.
25. Meyer MR, Peters FT, Maurer HH. Automated mass spectral deconvolution and identification system for GC-MS screening for drugs, poisons, and metabolites in urine. Clin Chem. 2010;56:575–84.
26. Wissenbach DK, Ding J, Neuwirth T, Weber AA, Peters FT. Implementation of macro-based extracted ion chromatograms for “AMDIS Software.” TIAFT. 2020;50:13–6.
27. Maurer HH, Pfleger K, Weber AA. Mass spectral data of drugs, poisons, pesticides, pollutants and their metabolites. 5th ed. Weinheim: Wiley-VCH; 2016.
28. Maurer HH, Meyer MR, Helfer AG, Weber AA. Maurer/Meyer/Helfer/Weber MMHW LC-HR-MS/MS library of drugs, poisons, and their metabolites. Weinheim: Wiley-VCH; 2018.
29. Maurer HH, Pfleger K, Weber AA. Mass spectral library of drugs, poisons, pesticides, pollutants and their metabolites. 5th ed. Weinheim: Wiley-VCH;2016.
30. Oberacher H, Reinstadler V, Kreidl M, Stravs MA, Hollender J, Schymanski EL. Annotating nontargeted LC-HRMS/MS data with two complementary tandem mass spectral libraries. Metabolites. 2018;9:3–15.
31. Partridge E, Trobbiani S, Stockham P, Scott T, Kostakis C. A validated method for the screening of 320 forensically significant compounds in blood by LC/QTOF, with simultaneous quantification of selected compounds. J Anal Toxicol. 2018;42:220–31.
32. Grapp M, Kaufmann C, Streit F, Binder L. Systematic forensic toxicological analysis by liquid chromatography-quadrupole-time-of-flight mass spectrometry in serum and comparison to gas chromatography-mass spectrometry. Forensic Sci Int. 2018;287:63–73.
33. Pasin D, Cawley A, Bidny S, Fu S. Characterization of hallucinogenic phenylamines using high-resolution mass spectrometry for non-targeted screening purposes. Drug Test Anal. 2017;9:1620–9.
34. Danso D, Langman LJ, Jannetto PJ. Targeted opioid screening assay for pain management using high-resolution mass spectrometry. Methods Mol Biol. 1872;2019:41–50.
35. Armenian P, Darraçq M, Gevorkyan J, Clark S, Kaye B, Brandenhoff NP. Intoxication from the novel synthetic cannabinoids AB-PINACA and ADB-PINACA: a case series and review of the literature. Neuropharmacology. 2018;134:82–91.
36. Joyce T, Sidibe J, Deglon J, Karmime A, Sporck F, Widmer C, et al. Liquid chromatography-high resolution mass spectrometry for broad-spectrum drug screening of dried blood spot as microsampling procedure. Anal Chim Acta. 2019;1063:110–6.
37. Fabresse N, Larabi IA, Stratton T, Mistrik R, Pfau G, Lorin de la Grandmaison G, et al. Development of a sensitive untargeted liquid chromatography-high resolution mass spectrometry screening devoted to hair analysis through a shared MS2 spectra database: a step toward early detection of new psychoactive substances. Drug Test Anal. 2019;11:697–708.
38. Alcantara-Duran J, Moreno-Gonzalez D, Beneito-Cambra M, Garcia-Reyes JF. Dilute-and-shoot coupled to nanoflow liquid chromatography-high resolution mass spectrometry for the determination of drugs of abuse and sport drugs in human urine. Talanta. 2018;182:218–24.
39. Salgueiro-Gonzalez N, Castiglioni S, Gracia-Lor E, Bijlsma L, Celma A, Bagnati R, et al. Flexible high resolution-mass spectrometry approach for screening new psychoactive substances in urban wastewater. Sci Total Environ. 2019;689:679–90.
40. Mollerup CB, Mardal M, Stravs MA, Linnet K, Barron LP. Prediction of collision cross section and retention time for broad scope screening in gradient reversed-phase liquid chromatography-ion mobility-high resolution accurate mass spectrometry. J Chromatogr A. 2018;1542:82–8.
41. Michely JA, Meyer MR, Maurer HH. Paper spray ionization coupled to high resolution tandem mass spectrometry for comprehensive urine drug testing in comparison to liquid chromatography-coupled techniques after urine precipitation or dried urine spot workup. Anal Chem. 2017;89:11779–86.
42. Michely JA, Meyer MR, Maurer HH. Power of Orbitrap-based LC-high resolution-MS/MS for comprehensive drug testing in urine with or without conjugate cleavage or using dried urine spots after on-spot cleavage in comparison to established LC-MS(n) or GC-MS procedures. Drug Test Anal. 2018;10:158–63.
43. McKenna J, Jett R, Shanks K, Manicke NE. Toxicological drug screening using paper spray high-resolution tandem mass spectrometry (HR-MS/MS). J Anal Toxicol. 2018;42:300–10.
44. Duvivier WF, van Putten MR, van Beek TA, Nielen MW. (Un)targeted scanning of locks of hair for drugs of abuse by direct analysis in real time-high-resolution mass spectrometry. Anal Chem. 2016;88:2489–96.
45. Jagerdeo E, Wriston A. Rapid analysis of forensic-related samples using two ambient ionization techniques coupled to high-resolution mass spectrometers. Rapid Commun Mass Spectrom. 2017;31:782–90.
46. Sofalvi S, Lavins ES, Kaspar CK, Michel HM, Mitchell-Mata CL, Huetsi MA, et al. Development and validation of an LC-MS-MS method for the detection of 40 benzodiazepines and three Z-drugs in blood and urine by solid-phase extraction. J Anal Toxicol. 2020;44:708–17.
47. Caspar AT, Kollas AB, Maurer HH, Meyer MR. Development of a quantitative approach in blood plasma for low-dosed hallucinogens and opioids using LC-high resolution mass spectrometry. Talanta. 2018;176:635–45.
48. Thomas A, Yang R, Petring S, Bally L, Thevis M. Simplified quantification of insulin, its synthetic analogs and C-peptide in human plasma by means of LC-HRMS. Drug Test Anal. 2020;12:382–90.
49. Thomas A, Thevis M. Recent advances in the determination of insulin from biological fluids. Adv Clin Chem. 2019;93:115–67.
50. Gao X, Li H, Li H, Dong S, Chu J, Guo H, et al. Sensitive determination of nine anticoagulant rodenticides in blood by high-resolution mass spectrometry with supported liquid extraction pretreatment. Forensic Sci Int. 2018;292:39–44.
51. Kronstrand R, Forsman M, Roman M. Quantitative analysis of drugs in hair by UHPLC high resolution mass spectrometry. Forensic Sci Int. 2018;283:9–15.
52. Meyer MR. Toxicokinetics of NPS – update 2017. Handb Exp Pharmacol. 2018;252:441–59.
53. Richter LH, Flockerzi V, Maurer HH, Meyer MR. Pooled human liver preparations, HepaRG, or HepG2 cell lines for metabolism studies of new psychoactive substances? A study using MDMA, MDBD, butylone, MDPBB, MDPV, MDPB, 5-MAPB, and 5-API as examples. J Pharm Biomed Anal. 2017;143:32–42.
54. Diao X, Huestis MA. New synthetic cannabinoids metabolism and strategies to best identify optimal marker metabolites. Front Chem. 2019;7:109.
55. Diao X, Huestis MA. Approaches, challenges, and advances in metabolism of new synthetic cannabinoids and identification of optimal urinary marker metabolites. Clin Pharmacol Ther. 2017;101:239–53.
56. Ellefsen KN, Concheiro M, Huestis MA. Synthetic cathinone pharmacokinetics, analytical methods, and toxicological findings from human performance and postmortem cases. Drug Metab Rev. 2016;48:237–65.
57. Meyer MR. New psychoactive substances: an overview on recent publications on their toxicodynamics and toxicokinetics. Arch Toxicol. 2016;90:2421–44.
58. Naz S, Gallart-Ayala H, Reinke SN, Mathon C, Blankley R, Chaleckis R, et al. Development of a liquid chromatography-high resolution mass spectrometry metabolomics method with high specificity for metabolite identification using all ion fragmentation acquisition. Anal Chem. 2017;89:7933–42.
59. Pezzatti J, Boccard J, Codesido S, Gagnebin Y, Joshi A, Picard D, et al. Implementation of liquid chromatography-high resolution mass spectrometry methods for untargeted metabolomic analyses of biological samples: a tutorial. Anal Chim Acta. 2020;1105:28–40.
60. Manier SK, Meyer MR. Current situation of the metabolomics techniques used for the metabolism studies of new psychoactive substances. Ther Drug Monit. 2020;42:93–7.
61. Manier SK, Keller A, Schaper J, Meyer MR. Untargeted metabolomics by high resolution mass spectrometry coupled to normal and reversed phase liquid chromatography as a tool to study the in vitro biotransformation of new psychoactive substances. Sci Rep. 2019;9:2741.

62. Brockbals L, Kraemer T, Steuer AE. Analytical considerations for postmortem metabolomics using GC-high-resolution MS. Anal Bioanal Chem. 2020;412:6241–55.

63. Boxler MI, Schneider TD, Kraemer T, Steuer AE. Analytical considerations for (un)-targeted metabolomic studies with special focus on forensic applications. Drug Test Anal. 2019;11:678–96.

64. Steuer AE, Kaelin D, Boxler MI, Eisenbeiss L, Holze F, Vizeli P, et al. Comparative untargeted metabolomics analysis of the psychostimulants 3,4-methylenedioxy-methamphetamine (MDMA), amphetamine, and the novel psychoactive substance mephedrone after controlled drug administration to humans. Metabolites. 2020;10:306.

65. Steuer AE, Brockbals L, Kraemer T. Metabolomic strategies in biomarker research—new approach for indirect identification of drug consumption and sample manipulation in clinical and forensic toxicology? Front Chem. 2019;7:319.

66. Steuer AE, Raeber J, Steuer C, Boxler MI, Dombierer DA, Bosch OG, et al. Identification of new urinary gamma-hydroxybutyric acid markers applying untargeted metabolomics analysis following placebo-controlled administration to humans. Drug Test Anal. 2019;11:813–23.

67. Boxler MI, Streun GL, Liechti ME, Schmid Y, Kraemer T, Steuer AE. Human metabolome changes after a single dose of 3,4-methylenedioxyamphetamine (MDMA) with special focus on steroid metabolism and inflammation processes. J Proteome Res. 2018;17(8):2900–7.

68. Mollerup CB, Rasmussen BS, Johansen SS, Mardal M, Linnet K, Dalsgaard PW. Retrospective analysis for valproate screening targets with liquid chromatography-high resolution mass spectrometry with positive electrospray ionization: an omics-based approach. Drug Test Anal. 2019;11:730–8.

69. Piper T, Mehtling LM, Spottke A, Heidbreder A, Young P, Madea B, et al. Potential of GHB phase-II-metabolites to complement current approaches in GHB post administration detection. Forensic Sci Int. 2017;279:157–64.

70. Eisenbeiss L, Binz TM, Baumgartner MR, Kraemer T, Steuer AE. Cheating on forensic hair testing? Detection of potential biomarkers for cosmetically altered hair samples using untargeted metabolomics. Analyst. 2020;145:6586–99.

71. Eisenbeiss L, Binz TM, Baumgartner MR, Steuer AE, Kraemer T. A possible new oxidation marker for hair adulteration: detection of PTECA (1H-pyrole-2,3,4,5-tetracarboxylic acid) in bleached hair. Drug Test Anal. 2020;12:230–8.

72. Steuer AE, Kamber D, Kraemer T. Evaluation of endogenous urinary biomarkers for indirect detection of urine adulteration attempts by five different chemical adulterants in mass spectrometry methods. Drug Test Anal. 2019;11:638–48.

73. Steuer AE, Arnold K, Kamber D, Kraemer T. Suitability evaluation of new endogenous urinary biomarkers for the identification of nitrite-based urine adulteration in mass spectrometry methods. Drug Test Anal. 2019;11:230–9.

74. Steuer AE, Arnold K, Schneider TD, Poetzsch M, Kraemer T. A new metabolomics-based strategy for identification of endogenous markers of urine adulteration attempts exemplified for potassium nitrate. Anal Bioanal Chem. 2017;409:6235–44.

75. Manier SK, Wagmann L, Flockerzi V, Meyer MR. Toxicometabolomics of the new psychoactive substances aPBP and alpha-PEP studied in HepaRG cell incubates by means of untargeted metabolomics revealed unexpected amino acid adducts. Arch Toxicol. 2020;94:2047–59.

76. Narduzzi L, Dervilly G, Audran M, Le Bizec B, Buissen C. A role for metabolomics in the antidoping toolbox? Drug Test Anal. 2020;12:677–90.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.