# New Psychoactive Substances: Which Biological Matrix Is the Best for Clinical Toxicology Screening?

*Lea Wagmann, PhD, Cathy M. Jacobs, MSc, and Markus R. Meyer, PhD*



**Background:** Every year, more new psychoactive substances (NPSs) emerge in the market of the drugs of abuse. NPSs belong to various chemical classes, such as synthetic cannabinoids, phenethylamines, opioids, and benzodiazepines. The detection of NPSs intake using different types of biological matrices is challenging for clinical toxicologists because of their structural diversity and the lack of information on their toxicokinetics, including their metabolic fate.

**Methods:** PubMed-listed articles reporting mass spectrometry–based bioanalytical approaches for NPSs detection published during the past 5 years were identified and discussed. Furthermore, the pros and cons of using common biological matrices in clinical toxicology (CT) settings to screen for NPSs are highlighted in this review article.

**Results:** Twenty-six articles presenting multianalyte screening methods for use in the field of CT were considered. The advantages and disadvantages of different biological matrices are discussed with a particular view of the different analytical tasks in CT, especially emergency toxicology. Finally, an outlook introduces the emerging trends in biosamples used in CT, such as the exhaled breath.

**Conclusions:** Blood and urine represent the most common biological matrices used in a CT setting; however, reports concerning NPSs detection in alternative matrices are also available. Noteworthy, the selection of the biological matrix must depend on the clinician's enquiry because the individual advantages and disadvantages must be considered.

**Key Words:** new psychoactive substances, drugs of abuse, mass spectrometry, clinical toxicology

(*Ther Drug Monit* 2022;44:599–605)

## INTRODUCTION

Detection of emerging drugs of abuse is essential in clinical toxicology (CT) to confirm their intake, for example, in case of overdoses followed by acute intoxications. The new psychoactive substances (NPSs), which are brought on the market as substitutes for the traditional drugs of abuse, are particularly challenging analytical toxicology since 2005. Their number is increasing every year, and at the end of 2020, the European Monitoring Centre for Drugs and Drug Addiction, EMCDDA, witnessed over 800 NPS, 46 of which were first reported in Europe in 2020.[1] Although the consumption levels of NPSs are expected to be low in Europe, 2 thirds of the countries reported health concerns because of NPSs, especially for their high-risk drug users.[1] This concern seems justified by the high number of reported NPS-related seizures to the EU Early Warning System every year.[1]

Thus, keeping pace with the development of drugs in the abuse market is an important task in analytical toxicology. However, the demands in different areas, such as CT, forensic toxicology, and doping control, are very specific. According to Flanagan et al,[2] CT is often considered as "emergency and general hospital toxicology, including poison screening." This statement defines 2 NPS-related working areas in CT. First, emergency toxicology analyses are performed whenever the patient's life is endangered and the intake of drugs of abuse is suspected. Such acute intoxication may be caused by combining or overdosing the traditional drugs of abuse and the NPS. Second, abstinence control analyses were conducted to rule out chronic abuse in the context of general hospital toxicology. In this context, chronic abuse may be defined as the continuous and repeated intake of one or more drugs of abuse, including NPSs, because their intake does not necessarily cause immediate life-threatening conditions or acute intoxication. Screening of drug abuse to confirm abstinence is also a part of CT and will be considered in this review article. This includes CT screenings to detect chronic NPSs abuse or confirm the absence of NPSs, for example, in psychiatric outpatients. In both cases, CT analyses aim to answer the question of whether the patient had recently ingested any drugs of abuse.

In case of emergency toxicology, the results of analysis should be available in a reasonable time, allowing clinicians to identify the causes for the patient's condition and to weigh adequate treatment options. CT analyses are often qualitative. However, quantitative analyses can be of particular interest if their results may change the treatment regimen for the patient, for example, hemodialysis in addition to antidote administration in case of methanol poisoning.[3] Biological matrices used for CT screenings are usually blood and urine but can be limited to blood alone, for example, in case of anuric patients.

Previously, CT was performed using immunoassays and thin-layer chromatography. Currently, mass spectrometry coupled with gas or liquid chromatography is considered the gold standard.[4] The commonly used mass analyzers range

Received for publication October 19, 2021; accepted December 9, 2021.

From the Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Center for Molecular Signaling (PZMS), Saarland University, Homburg, Germany.

The authors declare no conflict of interest.

Correspondence: Lea Wagmann, PhD, Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Center for Molecular Signaling (PZMS), Saarland University, Kirrberger Straße Building 46, D-66421 Homburg, Germany (e-mail: lea.wagmann@uks.eu).

from single quadrupoles to high-resolution instruments that combine different mass analyzers. Regardless of the instrument type, analytical information about the screened compounds needs to be available. This usually includes the retention time or the retention index and the mass spectrum, each recorded under defined conditions. Thus, emerging compounds, such as NPSs, need to be characterized, and the data made publicly available as soon as possible. This can be achieved by scientific publications for selected compounds[5] or in the form of reference databases.[6–8] It should be mentioned that not only the analytical data of the unchanged parent compounds are needed but also the information about the renally excreted metabolites because they may be the main targets in urine analysis. Recently, new developments to complement the mentioned strategies have been introduced, such as the activity-based assays.[9] However, despite using immunoassays, which were found to be inappropriate for the detection of NPSs,[10] or activity-based assays as initial screening strategies, findings usually need to be confirmed by mass spectrometry.

This review article discusses the advantages and disadvantages of human sample matrices, which are usually available in a CT setting. Different analytical tasks will be considered, with a focus on NPSs detection in emergency toxicology, drug abuse screening, and multianalyte methods.

## METHODS

Relevant articles were identified through a literature search of PubMed (https://pubmed.ncbi.nlm.nih.gov). Scientific articles published between April 01, 2016, and March 31, 2021, were selected using the following keywords "NPSs" or "novel psychoactive substances" in combination with "screening" and "mass spectrom*" in the title or the abstract ((((NPS[Title/Abstract]) OR (novel psychoactive substances[Title/Abstract])) AND (screening[Title/Abstract])) AND (mass spectrom*[Title/Abstract])) AND ((("2016/04/01"[Date—Publication]: "2021/03/31"[Date—Publication])). A total of 145 articles were identified, but only 26 were written in English and presented multianalyte screening methods to be used in the field of CT and were thus considered in detail for this review article.

## RESULTS AND DISCUSSION

CT screenings for the detection of NPSs intake may be requested not only in case of emergencies but also as abstinence control to rule out the chronic consumption of drugs of abuse. CT screening can be performed in readily available human biosamples, such as blood, urine, or oral fluid (OF).[11] However, in case of emergencies, physicians may not have access to all these matrices. Urine may be difficult to obtain if a patient is anuric or uncooperative. Furthermore, the sampling of OF is difficult in comatose patients or patients with anticholinergic syndrome. Owing to different preconditions and requirements, a distinction should be made between CT screenings to detect acute NPSs intoxications leading to emergencies and life-threatening conditions and those to detect chronic NPSs abuse or confirm abstinence.

### Detection of Acute New Psychoactive Substances Intoxications Using Blood or Urine

In general, blood and urine are the biosamples of choice to perform CT screenings in case of acute intoxications.[12] Table 1 presents an overview of recently published analytical methods covering multiple NPSs and/or their metabolites in blood and urine. The latter is still preferred as a biological matrix for screening purposes.[11] Large volumes of urine can be obtained noninvasively and the drugs (of abuse), and/or their metabolites are concentrated in urine.[11] Furthermore, the detection window is prolonged compared with blood as a sample matrix. However, information on the urinary excretion patterns of NPSs is required first.[10,11] Even sophisticated techniques such as gas chromatography–mass spectrometry (GC-MS), liquid chromatography–tandem mass spectrometry, or liquid chromatography–high-resolution tandem mass spectrometry are not able to detect NPSs intake if the reference mass spectra of newly emerging NPSs or their metabolites are not included in the mass spectral library.[11] Staeheli et al[13] addressed this problem by performing in vitro incubations using human liver microsomes to identify the metabolites and established a screening method for 75 synthetic cannabinoids in addition to their metabolites in urine. Owing to their extensive metabolism, most synthetic cannabinoids are not detectable unmetabolized in urine screenings. For NPSs, screening methods must focus on the excreted metabolites. However, the interpretation of analytical data regarding the metabolites may be complicated because some NPSs share common metabolites.[14] Apart from the human liver microsomes, incubations with human hepatocytes or the fungus *C. elegans*, in vivo models, such as rats or zebrafish, and in silico predictions were used to elucidate the metabolism of synthetic cannabinoids in the past.[14]

In contrast to synthetic cannabinoids, the parent compounds of stimulants such as synthetic cathinones and piperazines are usually detectable in the human urine.[11] Because urine is mostly free of proteins or lipids, simple sample preparation procedures can be applied.[11] Gerace et al[15] used a liquid–liquid extraction followed by derivatization for the determination of 19 stimulants in urine within a run time of 10 minutes using GC-MS. Stephanson et al[16] used a 5-fold dilution of urine for screening 120 NPSs using LC-HRMS/MS, and Kennedy et al[17] described a paper spray–based method for the quantification of 7 synthetic opioids in urine, requiring no sample preparation at all.

However, not only urine but also blood can be used for the fast screening of NPSs. Adamowicz et al described different screening methods for 80 stimulants,[18] 38 synthetic opioids,[19] and 143 NPSs of different classes[20] in blood. Furthermore, Mercieca et al[21] presented a method for the detection and quantification of 22 stimulants in whole blood and urine. Ares-Fuentes et al[22] developed an analytical strategy for the quantification of 5 designer benzodiazepines in plasma. More details concerning the sample preparation procedures, instrumentation, and detection methods are presented in Table 1.

Some of the presented methods allow for the quantification of NPSs in blood and urine samples (see Table 1). Quantitative analysis requires the availability of

**TABLE 1.** Biosamples, Experimental Setup, and Highlights of Multianalyte Approaches Covering NPSs Suitable for Clinical Toxicological Screening in Case of Acute Intoxications

| Covered NPSs | Biosample | Sample Preparation | Instrumentation | Detection Mode | Highlights | Reference |
|---|---|---|---|---|---|---|
| 75 synthetic cannabinoids and metabolites | U | Enzymatic hydrolysis + SALLE | LC-MS/MS | MRM | Analysis of authentic samples<br>In vitro assay for metabolite identification | Staeheli et al[13] |
| 19 stimulants | U | LLE + derivatization | GC-MS | SIM | Analysis of authentic samples | Gerace et al[15] |
| 35 metabolites of synthetic cannabinoids | U | Enzymatic hydrolysis + SPE | LC-HRMS/MS | Full scan + targeted MS/MS for confirmation | Analysis of authentic samples<br>Use of a pipetting robot | Gundersen et al[38] |
| 11 stimulants | U | Porous membrane protected MIP μ-SPE | LC-MS/MS | MRM | Method can be adapted for other body fluids | Sanchez-Gonzalez et al[39] |
| 7 synthetic opioids | U | None: 10 μL urine on paper spray cartridge | LC-MS/MS and LC-HRMS/MS | MRM or full scan + data-dependent MS/MS | No sample preparation<br>Paper spray ionization | Kennedy et al[17] |
| 120 NPSs | U | Dilution | LC-HRMS/MS | Full scan and PRM | Analysis of authentic samples<br>Short run time (6 min) | Stephanson et al[16] |
| 38 synthetic opioids | WB | LLE | LC-MS/MS | Dynamic MRM | Analysis of authentic samples<br>New compounds can be integrated | Adamowicz et al[19] |
| 29 synthetic cannabinoids and metabolites | WB | SLE | LC-MS/MS | MRM | Analysis of authentic samples<br>Short run time (9 min) | Ong et al[40] |
| 80 stimulants | WB | Precipitation | LC MS/MS | Dynamic MRM | New compounds can be integrated | Adamowicz and Tokarczyk[18] |
| 22 stimulants | WB, U | Derivatization, DLLME | GC-MS | Scan mode (*m/z* 50–390) | Analysis of authentic samples<br>Quantitative method | Mercieca et al[21] |
| 45 synthetic opioids | WB | Precipitation | LC-HRMS/MS | DIA in $MS^E$ mode | Analysis of authentic samples<br>New compounds can be integrated<br>Targeted screening | Noble et al[41] |
| 10 stimulants and 1 synthetic opioid | P, WB | PALME | LC-MS/MS | SRM | Quantitative method | Vardal et al[42] |
| 11 designer benzodiazepines | WB | Precipitation | LC-MS/MS | Targeted and nontargeted screening | Analysis of authentic samples<br>Automated sample preparation<br>Tentative identification of compounds not included in the initial screening | Mollerup et al[43] |
| Library based | P | Precipitation | LC-HRMS/MS | Full scan + targeted MS/MS | New compounds can be integrated<br>Quantitative method | Montesano et al[44] |
| 33 stimulants and 28 synthetic cannabinoids | WB | Precipitation | LC-MS/MS | Dynamic MRM | Analysis of authentic samples | Vaiano et al[45] |
| 103 stimulants and 34 synthetic cannabinoids | WB | Precipitation | LC-MS/MS | Dynamic MRM | Analysis of authentic samples<br>New compounds can be integrated | Adamowicz and Tokarczyk[20] |

(*continued on next page*)

**TABLE 1.** (*Continued*) Biosamples, Experimental Setup, and Highlights of Multianalyte Approaches Covering NPSs Suitable for Clinical Toxicological Screening in Case of Acute Intoxications

| Covered NPSs | Biosample | Sample Preparation | Instrumentation | Detection Mode | Highlights | Reference |
|---|---|---|---|---|---|---|
| Library based | S | LLE | LC-HRMS/MS | DIA in $MS^E$ mode | Analysis of authentic samples<br>Comparison to GC-MS screening | Grapp et al[46] |
| 5 designer benzodiazepines | P | MEPS | LC-MS/MS | MRM | Analysis of authentic samples<br>Quantitative method<br>Short run time (5 min) | Ares-Fuentes et al[22] |

U, urine; WB, whole blood; P, plasma; S, serum; LLE, liquid–liquid extraction; SPE, solid-phase extraction; SALLE, salting-out LLE; MIP, molecularly imprinted polymers; SLE, supported liquid extraction; DLLME, dispersive liquid–liquid microextraction; US-DLLME, ultrasound-assisted DLLME; PALME, parallel artificial liquid membrane extraction; MEPS, microextraction by packed sorbent; GC-MSLC-MS/MS, liquid chromatography–tandem mass spectrometry; MRM, multiple reaction monitoring; SIM, selected ion monitoring; PRM, parallel reaction monitoring; SRM, selected reaction monitoring; DIA, data-independent acquisition.

reference standards, which are expensive and are usually not available for all NPSs and/or metabolites.[11] Moreover, only scarce information on the blood concentrations after recreational use of NPSs is available. Studies have demonstrated that NPSs blood concentrations do not always reflect the clinical symptoms or the severity of intoxication. Observed concentrations vary widely and can have overlapping ranges for fatal and nonfatal cases.[23,24] Therefore, the determination of NPSs blood concentrations is expected to be more useful in forensic toxicology to evaluate impairments or fatalities than in a CT setting. The interpretation of urine concentrations is even more challenging because excretion depends on several factors such as the kidney and liver function and the metabolic capacity of the patient. Only normalization to urinary creatinine concentrations may allow a certain degree of interindividual comparability. Another fact that seems to be neglected in a CT setting is the separation of optical isomers, although their identification may be an important issue in forensic toxicology owing to differences in the legal status or toxicity.[10]

**TABLE 2.** Biosamples, Experimental Setup, and Highlights of Multianalyte Approaches Covering NPSs Suitable for Clinical Toxicological Screening to Detect Chronic NPSs Abuse

| Covered NPSs | Biosample | Sample Preparation | Instrumentation | Detection Mode | Highlights | Reference |
|---|---|---|---|---|---|---|
| 87 NPSs | OF | LLE | LC-MS/MS | MRM | Analysis of authentic samples | da Cunha et al[25] |
| 36 synthetic cannabinoids, 12 synthetic opioids, 29 stimulants, and 10 metabolites | OF, WB, U | Dilution (OF, U), precipitation (WB) | LC-MS/MS | MRM | Analysis of authentic samples | Trana et al[26] |
| 10 synthetic cannabinoids | OF | Denaturation | LC-MS/MS | MRM | Quantitative method | Calo et al[47] |
| 10 synthetic cannabinoids and 1 stimulant | OF | MEPS | DESI-MS | Point to point oscillating acquisition mode and full scan | GC-MS (SIM) confirmation method | Bianchi et al[48] |
| 10 stimulants | OF | US-DLLME | LC-MS/MS | MRM | Analysis of authentic samples<br>Short run time (5 min) | Fernandez et al[49] |
| 17 synthetic cannabinoids and metabolites, and 13 stimulants | OF | MEPS | LC-MS/MS | MRM | New compounds can be integrated | Rocchi et al[27] |
| 11 stimulants | OF | MEPS | LC-MS/MS | MRM | Analysis of authentic samples<br>Quantitative method<br>Short run time (8 min) | Ares et al[50] |
| 37 stimulants and metabolites | DBS | Sonification, LLE | LC-MS/MS | MRM | Quantitative method | Wang et al[28] |

WB, whole blood; U, urine; MEPS, microextraction by packed sorbent; LLE, liquid–liquid extraction; US-DLLME, ultrasound-assisted dispersive liquid–liquid microextraction; LC-MS/MS, liquid chromatography–tandem mass spectrometry; DESI-MS, desorption electrospray ionization–mass spectrometry; MRM, multiple reaction monitoring; SIM, selected ion monitoring.

## Detection of Chronic New Psychoactive Substances Abuse Using Oral Fluid or Dried Blood Spots

CT analyses to detect chronic NPSs abuse or demonstrate abstinence are usually not as urgent as those used for the detection of acute intoxication. The analysis results are expected within days rather than within a few hours. For this purpose, alternative matrices, such as OF, or alternative sampling strategies, such as dried blood spots (DBSs), can be used in addition or as an alternative to traditional blood and urine specimens. Other alternative matrices, such as hair or nails, which can prove drug intake a long time ago, are usually only of interest in forensic toxicology, but not in CT. However, all the methods presented for the detection of acute NPSs intoxication using blood or urine may also be suitable for the detection of chronic NPSs abuse. In addition, Table 2 presents an overview of recently published analytical methods covering multiple NPSs and/or their metabolites in OF or DBSs.

OF can be noninvasively collected under observation by nonmedical staff, and the determined concentrations better reflect blood concentrations than urine.[25] Drugs with basic properties can concentrate in the OF, leading to improved sensitivity for NPSs detection. By contrast, acidic drugs show lower concentrations in OF than in blood.[25] However, OF has some drawbacks such as a smaller collection volume in comparison with urine and a lack of correlation with blood concentrations immediately after oral drug ingestion or inhalation because of contamination in the mouth.[25] Furthermore, OF only provides evidence of recent drug use with a shorter detection window than urine.[25] Trana et al[26] developed a screening method for 77 NPSs and 10 metabolites of different classes in OF, blood, and urine. da Cunha et al[25] presented a screening method for 87 NPSs of different classes, and Rocchi et al[27] described a method for simultaneous screening and quantification of 31 NPSs of different classes in OF. More details concerning sample preparation procedures, instrumentation, and detection methods are presented in Table 2.

DBSs require only a small amount of blood and are excellent for the shipment or storage of biological samples given their small dimensions, and the fact that they frequently do not require refrigeration.[28] Furthermore, DBSs sampling can enhance the stability of compounds, which can play an increasingly important role in the analysis of NPSs and their metabolites in blood.[28] Wang et al[28] developed a screening and quantification method for 37 stimulants and metabolites in DBSs, proving the suitability of this sampling strategy for CT analysis.

## GAP ANALYSIS

Clinical toxicologists face several difficulties in attempting to detect NPSs in human biosamples. The first stumbling block may be the increasing complexity of the NPSs market, which is characterized by the rapid emergence of new substances. In 2014, Brandt et al[29] called the continuous circumvention of existing legislations, to market new abused drugs, a "cat and mouse game." The number of NPSs increased from less than 200 substances over the period 2005–2009 to 950 substances by the end of 2019. Authorities have recently identified more than 3 times as many NPSs worldwide because psychoactive substances are under international control.[30] Continuous market surveillance is essential. However, the analytical characterization of new substances followed by metabolism studies and implementation of potential screening targets in mass spectral libraries is time consuming and may exceed the half-life of a substance in the market. The situation becomes even more challenging because of local trends such as the opioid crisis in North America driven by synthetic opioids.[31] A potential solution to this challenge is the workflow described by Garneau et al,[32] which includes all steps from the detection of a regional NPSs threat to its implementation into a detection method.

Nevertheless, other factors influence the successful detection of NPSs in human biosamples. As previously mentioned, owing to the extensive metabolism of some NPSs classes, such as synthetic cannabinoids, parent compounds are rarely detectable in urine. In addition, the sample preparation has a significant impact. In GC-MS analysis, conjugate cleavage and derivatization are often inevitable.[8] In LC-based analysis, glucuronides and other phase II metabolites may serve as additional screening targets,[6,7] whereas conjugate cleavage during sample preparation may enhance the concentration of the corresponding phase I metabolites. The assumed timing of drug intake also influences the selection of a suitable sample matrix. Blood and OF usually reflect recent drug intake while urine allows for a broader detection window, which is crucially influenced by toxicokinetics. Nevertheless, NPSs that are consumed in low doses, such as LSD derivatives or synthetic opioids, require more laborious sample preparation procedures, such as solid-phase extraction.[5]

Gastric contents, which are sometimes available as a specimen, may also be considered as biosample in emergency toxicology. However, limited knowledge is available concerning the detectability of NPSs in gastric content during CT screenings. The benefit is usually a high concentration of analytes; however, this may lead to contamination of the sample preparation setup and analytical instruments. Thus, analysis of gastric content should not be a solid part of any CT screening but should be considered in certain situations. This is also true for unidentified powders or herbal mixtures found together with the patient. Their analysis cannot prove intake and therefore cannot replace the analysis of human biosamples but suggests a suitable biosample preparation and analysis procedure. However, the risk of contamination of analytical instruments owing to high concentrations must be considered.

The detection of prenatal exposure to drugs of abuse in newborns is also a task in CT. Consumption of drugs of abuse during pregnancy increases the risk of premature delivery, fetal growth restriction, and neonatal abstinence syndrome.[33] Meconium has been used for decades to document prenatal exposure to abused drugs, but limited data are available about prenatal exposure to NPSs.[33] Recently, López-Rabuñal et al developed an analytical procedure for the determination of 137 NPSs and other drugs of abuse in meconium, consisting of homogenization, solid-phase extraction, and LC-HRMS/

MS analysis and reported acetylfentanyl detection in 2 of 30 meconium specimens.[34]

## CONCLUDING AUTHOR OPINION

As previously discussed, the selection of a biological matrix depends on the clinician's enquiry. In emergency toxicology, urine analysis may be sufficient to detect recent drug intake. The drawback is that it cannot be concluded whether the patient is under acute influence because the compound can still be present in urine after it has been removed from the blood. Analyzing blood samples allows detecting whether drugs are still present in the systemic circulation and may still have an effect. This interpretation is also dependent on reference "therapeutic" blood concentrations, which are usually not available for NPSs. One drawback of analyzing blood is the lower analyte concentration, in contrast to urine, and the absence of metabolites confirming the presence of the NPS.

In abstinence screening, urine may be the best choice because the main question is not whether the patient is under acute influence rather than whether the patient has recently ingested a drug of abuse. An alternative may be OF, with the limitation that usually lower concentrations are expected, and that not all drugs are excreted into this matrix.

## OUTLOOK

The increasing number of NPSs available for drugs in the abuse market calls for selective, sensitive, and flexible bioanalytical procedures. MS-based analysis strategies fulfil these needs, as evidenced by the high number of studies published during the past 5 years. However, CT laboratories need high economic resources to keep pace with the emergence of NPSs for drugs in the abuse market. These resources include analytical instrumentation, reference standards, metabolism models, and experienced personnel to steadily update bioanalytical procedures. Therefore, future implementation of a few reference laboratories for NPSs testing per country may be a promising option.

Data handling is also expected to gain even more importance in the coming years because complex, high-resolution instruments collect enormous amounts of data. Sophisticated workflows, including highly sensitive MS measurements and high-end data analysis, may expand the human sample matrices that can be used to successfully detect NPSs in CT. Exhaled breath may also be used as an alternative matrix to detect chronic NPSs abuse or demonstrate abstinence. Beck et al demonstrated the suitability of exhaled breath combined with liquid chromatography–tandem mass spectrometry analysis to detect the traditional drugs of abuse,[35,36] but data on NPSs detection are still missing. Another example is the analysis of wastewater for wastewater-based epidemiology. Such approaches are currently mainly used for the traditional drugs of abuse because low dosages and flexible NPSs consumption patterns pose limitations. However, the results of wastewater analysis could help detect current and regional NPSs trends.[37] This knowledge may be beneficial for clinical toxicologists to continuously adapt their bioanalytical strategies in the future.



## ACKNOWLEDGMENTS

*The authors thank Clémentine Richter and Matthias J. Richter for proofreading the manuscript.*

## REFERENCES

1. EMCDDA. *European Drug Report 2020*. Publications of of the European Union; 2020. Available at: http://www.emcdda.europa.eu/edr2020_en. Accessed October 08, 2021.
2. Flanagan RJ, Taylor A, Watson ID, et al. Analytical toxicology: overview. In: *Fundamentals of Analytical Toxicology*. Chichester, United Kingdom: John Wiley & Sons Ltd., 2008:1–19.
3. Brent J, Burkhart K, Dargan P, et al. *Critical Care Toxicology*. Basel, Switzerland: Springer International Publishing, 2017.
4. 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.
5. Wagmann L, Richter LHJ, Kehl T, et al. In vitro metabolic fate of nine LSD-based new psychoactive substances and their analytical detectability in different urinary screening procedures. *Anal Bioanal Chem.* 2019;411:4751–4763.
6. Maurer HH, Meyer MR, Helfer AG, et al. *Maurer/Meyer/Helfer/Weber MMHW LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites*. Weinheim, Germany: Wiley-VCH; 2018.
7. Maurer HH, Wissenbach DK, Weber AA. *Maurer/Wissenbach/Weber MWW LC-MSn Library of Drugs, Poisons, and Their Metabolites*. 2nd ed. Weinheim, Germany: Wiley-VCH; 2019.
8. Maurer HH, Pfleger K, Weber AA. *Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants/MA Spectral Library of Drugs, Poisons, Pesticides, Pollutants, and Their Metabolites 5e CD Software. 5*. Auflage ed. Weinheim, Germany: Wiley-VCH; 2016.
9. Cannaert A, Franz F, Auwärter V, et al. Activity-based detection of consumption of synthetic cannabinoids in authentic urine samples using a stable cannabinoid reporter System. *Anal Chem.* 2017;89:9527–9536.
10. Wagmann L, Maurer HH. Bioanalytical methods for new psychoactive substances. *Handb Exp Pharmacol.* 2018;252:413–439.
11. Peters FT. Recent developments in urinalysis of metabolites of new psychoactive substances using LC-MS. *Bioanalysis*. 2014;6:2083–2107.
12. Maurer HH. What is the future of (ultra) high performance liquid chromatography coupled to low and high resolution mass spectrometry for toxicological drug screening? *J Chromatogr A.* 2013;1292:19–24.
13. Staeheli SN, Veloso VP, Bovens M, et al. Liquid chromatography-tandem mass spectrometry screening method using information-dependent acquisition of enhanced product ion mass spectra for synthetic cannabinoids including metabolites in urine. *Drug Test Anal.* 2019;11:1369–1376.
14. Diao X, Huestis MA. New synthetic cannabinoids metabolism and strategies to best identify optimal marker metabolites. *Front Chem.* 2019;7:109.
15. Gerace E, Caneparo D, Borio F, et al. Determination of several synthetic cathinones and an amphetamine-like compound in urine by gas chromatography with mass spectrometry. Method validation and application to real cases. *J Sep Sci.* 2019;42:1577–1584.
16. Stephanson NN, Signell P, Helander A, et al. Use of LC-HRMS in full scan-XIC mode for multi-analyte urine drug testing—a step towards a "black-box" solution? *J Mass Spectrom.* 2017;52:497–506.
17. Kennedy JH, Palaty J, Gill CG, et al. Rapid analysis of fentanyls and other novel psychoactive substances in substance use disorder patient urine using paper spray mass spectrometry. *Rapid Commun MA Spectrom.* 2018;32:1280–1286.
18. Adamowicz P, Tokarczyk B. Screening analysis for designer stimulants by LC-MS/MS. *Methods Mol Biol.* 2019;1872:165–180.
19. Adamowicz P, Bakhmut Z, Mikolajczyk. A Screening procedure for 38 fentanyl analogues and five other new opioids in whole blood by liquid chromatography-tandem mass spectrometry. *J Appl Toxicol.* 2020;40:1033–1046.
20. Adamowicz P, Tokarczyk B. Simple and rapid screening procedure for 143 new psychoactive substances by liquid chromatography-tandem mass spectrometry. *Drug Test Anal.* 2016;8:652–667.

21. Mercieca G, Odoardi S, Cassar M, et al. Rapid and simple procedure for the determination of cathinones, amphetamine-like stimulants and other new psychoactive substances in blood and urine by GC-MS. *J Pharm Biomed Anal.* 2018;149:494–501.
22. Ares-Fuentes AM, Lorenzo RA, Fernandez P, et al. An analytical strategy for designer benzodiazepines and Z-hypnotics determination in plasma samples using ultra-high performance liquid chromatography/tandem mass spectrometry after microextraction by packed sorbent. *J Pharm Biomed Anal.* 2021;194:113779.
23. Adamowicz P. Blood concentrations of synthetic cannabinoids. *Clin Toxicol (Phila).* 2021;59:246–251.
24. Institoris L, Kovacs K, Sija E, et al. Clinical symptoms and blood concentration of new psychoactive substances (NPS) in intoxicated and hospitalized patients in the Budapest region of Hungary (2018-19). *Clin Toxicol (Phila).* 2022;60:18–24.
25. da Cunha KF, Oliveira KD, Huestis MA, et al. Screening of 104 new psychoactive substances (NPS) and other drugs of abuse in oral fluid by LC-MS-MS. *J Anal Toxicol.* 2020;44:697–707.
26. Trana AD, Mannocchi G, Pirani F, et al. A comprehensive HPLC-MS-MS screening method for 77 new psychoactive substances, 24 classic drugs and 18 related metabolites in blood, urine and oral fluid. *J Anal Toxicol.* 2020;44:769–783.
27. Rocchi R, Simeoni MC, Montesano C, et al. Analysis of new psychoactive substances in oral fluids by means of microextraction by packed sorbent followed by ultra-high-performance liquid chromatography-tandem mass spectrometry. *Drug Test Anal.* 2018;10:865–873.
28. Wang Y, Shi Y, Yu Y, et al. Screening of synthetic cathinones and metabolites in dried blood spots by UPLC-MS-MS. *J Anal Toxicol.* 2020;45:633–643.
29. Brandt SD, King LA, Evans-Brown M. The new drug phenomenon. *Drug Test Anal.* 2014;6:587–597.
30. UNODC. *World Drug Report 2020—Booklet 4. 2020*. Available at: https://wdr.unodc.org/wdr2020/index2020.html. Accessed October 08, 2021.
31. UNODC. The growing complexity of the opioid crisis. Global SMART Update Volume 24 2019. Available at: https://www.unodc.org/documents/scientific/Global_SMART-2020-Vol_24_web.pdf. Accessed October 08, 2021.
32. Garneau B, Desharnais B, Laquerre J, et al. A comprehensive analytical process, from NPS threat identification to systematic screening: method validation and one-year prevalence study. *Forensic Sci Int.* 2021;318:110595.
33. Lopez-Rabunal A, Di Corcia D, Amante E, et al. Simultaneous determination of 137 drugs of abuse, new psychoactive substances, and novel synthetic opioids in meconium by UHPLC-QTOF. *Anal Bioanal Chem.* 2021;413:5493–5507.
34. Carlier J, La Maida N, Di Trana A, et al. Testing unconventional matrices to monitor for prenatal exposure to heroin, cocaine, amphetamines, synthetic cathinones, and synthetic opioids. *Ther Drug Monit.* 2020;42:205–221.
35. Beck O, Leine K, Palmskog G, et al. Amphetamines detected in exhaled breath from drug addicts: a new possible method for drugs-of-abuse testing. *J Anal Toxicol.* 2010;34:233–237.
36. Beck O, Stephanson N, Sandqvist S, et al. Detection of drugs of abuse in exhaled breath from users following recovery from intoxication. *J Anal Toxicol.* 2012;36:638–646.
37. Bade R, Stockham P, Painter B, et al. Investigating the appearance of new psychoactive substances in South Australia using wastewater and forensic data. *Drug Test Anal.* 2019;11:250–256.
38. Gundersen POM, Spigset O, Josefsson M. Screening, quantification, and confirmation of synthetic cannabinoid metabolites in urine by UHPLC-QTOF-MS. *Drug Test Anal.* 2019;11:51–67.
39. Sanchez-Gonzalez J, Odoardi S, Bermejo AM, et al. HPLC-MS/MS combined with membrane-protected molecularly imprinted polymer micro-solid-phase extraction for synthetic cathinones monitoring in urine. *Drug Test Anal.* 2019;11:33–44.
40. Ong RS, Kappatos DC, Russell SGG, et al. Simultaneous analysis of 29 synthetic cannabinoids and metabolites, amphetamines, and cannabinoids in human whole blood by liquid chromatography-tandem mass spectrometry—a New Zealand perspective of use in 2018. *Drug Test Anal.* 2020;12:195–214.
41. Noble C, Weihe Dalsgaard P, Stybe Johansen S, et al. Application of a screening method for fentanyl and its analogues using UHPLC-QTOF-MS with data-independent acquisition (DIA) in MSE mode and retrospective analysis of authentic forensic blood samples. *Drug Test Anal.* 2018;10:651–662.
42. Vårdal L, Askildsen HM, Gjelstad A, et al. Parallel artificial liquid membrane extraction of new psychoactive substances in plasma and whole blood. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2017;1048:77–84.
43. Mollerup CB, Dalsgaard PW, Mardal M, et al. Targeted and non-targeted drug screening in whole blood by UHPLC-TOF-MS with data-independent acquisition. *Drug Test Anal.* 2017;9:1052–1061.
44. Montesano C, Vannutelli G, Gregori A, et al. Broad screening and identification of novel psychoactive substances in plasma by high-performance liquid chromatography-high-resolution mass spectrometry and post-run library matching. *J Anal Toxicol.* 2016;40:519–528.
45. Vaiano F, Busardò FP, Palumbo D, et al. A novel screening method for 64 new psychoactive substances and 5 amphetamines in blood by LC-MS/MS and application to real cases. *J Pharm Biomed Anal.* 2016;129:441–449.
46. Grapp M, Kaufmann C, Streit F, et al. 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.
47. Calò L, Anzillotti L, Maccari C, et al. Validation of a bioanalytical method for the determination of synthetic and natural cannabinoids (new psychoactive substances) in oral fluid samples by means of HPLC-MS/MS. *Front Chem.* 2020;8:439.
48. Bianchi F, Agazzi S, Riboni N, et al. Novel sample-substrates for the determination of new psychoactive substances in oral fluid by desorption electrospray ionization-high resolution mass spectrometry. *Talanta*. 2019;202:136–144.
49. Fernández P, Regenjo M, Ares A, et al. Simultaneous determination of 20 drugs of abuse in oral fluid using ultrasound-assisted dispersive liquid-liquid microextraction. *Anal Bioanal Chem.* 2019;411:193–203.
50. Ares AM, Fernández P, Regenjo M, et al. A fast bioanalytical method based on microextraction by packed sorbent and UPLC-MS/MS for determining new psychoactive substances in oral fluid. *Talanta*. 2017;174:454–461.