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Recent advances in the detection of drugs of abuse by Dried Blood Spots

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Abstract

Purpose of this review study is to sum up the main recent advances reported in the scientific literature about the detection of common drugs of abuse in biological samples upon their collection by Dried Blood Spot (DBS) devices. The most recent, innovative and fully validated methods for the qualitative and/or quantitative detection of common drugs of abuse are reported, including alprazolam, clonazepam, diazepam, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methyl- enedioxyethylamphetamine (MDEA), amphetamine, methamphetamine, cocaine, tetrahydrocannabinol (THC), 6-monoacetylmorphine, morphine, codeine, hydromorphone, hydrocodone, oxycodone, noroxycodone, tramadol, methadone, buprenorphine, fentanyl, ketamine and their respective metabolites and γ-hydroxybutyric acid (GHB). DBS proved to be extremely promising for routine analysis of forensic cases, although large-scale experiments on real samples need to be performed to confirm the emerging advantages of the technique and remove the potential limitations still affecting its widespread application.

Keywords drugs of abuse, DBS, LC-MS/MS, HRMS, green chemistry

Introduction

The Dried Blood Spot (DBS) microsampling technique was first proposed back in 1913 by Ivar Bang and used for glucose concentration monitoring (Bang I., 1913). In 1963, Guthrie and Susi successfully utilized dried blood sampling for newborn phenylketonuria screening (Guthrie & Susi, 1963). In the early 2000s, interest in DBS increased, resulting in the enactment of intensive scientific research and experimentation and finally leading to DBS recommendation as an accurate technique

for HIV and hepatitis B and C diagnosis by the World Health Organization (Biondi et al., 2019; Soulier et al., 2016). Currently, DBS is commonly used for newborn screening for a wide range of serious genetic and metabolic disorders (Carpenter & Wiley, 2002; Li & Tse, 2010) forensic screening of drugs of abuse and therapeutic drug monitoring (Corran et al., 2008; Lee & Li, 2014; Wilhelm et al., 2014). In 2021, the use of DBS also proved successful for COVID-19 epidemiological research using ELISA-based assays (Fontaine & Saez, 2021).

According to the global overview of drug demand and drug supply, issued by the United Nations Office on Drugs and Crime this year, more young people are using drugs compared with previous generations while cocaine production reaches its record high, and seizures of amphetamine and methamphetamine have dramatically increased (Global Overview Drug Demand Drug Supply, 2022). Furthermore, drug-related diseases and disorders keep rising among users while the illicit drug trade continues to hold back the economic and social development of several Countries. These combined plagues challenge the forensic toxicology laboratories to develop new, easy, accurate, and quick-turnaround methods to detect drugs of abuse in seizures and biological samples and help law officers to tackle these inherent ever-growing problems. Forensic toxicology involves a variety of professionals - clinicians, chemists, law officers - that must work together to achieve successful outcomes (Watterson, 2015). Within this context, forensic toxicology is traditionally distinguished between human-health toxicology, that analyses human specimens (blood, urine, hair, oral fluids) to assess whether drugs or toxins may have affected the donor's performances/abilities or may have caused him/her serious health disorders, and postmortem toxicology, whose goal is to assess whether drugs, alcohol, or toxins may have contributed or caused death of the investigated subject (Watterson, 2015). Moreover, *Stove et al.* aptly distinguished the DBS samples between the ones obtained from adult donors, addressed to investigate typical forensic toxicology inquiries, and those collected from newborns for the mere assessment of drug "exposure" prior to birth (Stove et al., 2012). Through the years, several publications appeared suggesting the use of DBS for drugs of abuse detection in a variety of circumstances, including performance tests, physical disorders diagnosis, abstinence compliance, early exposure assessment, and postmortem investigations. The classes of analytes that gained significant interest and whose positive detection has been demonstrated include benzodiazepines (e.g., alprazolam, clonazepam, diazepam,

3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methyl- enedioxyethylamphetamine (MDEA), amphetamine, methamphetamine, cocaine, tetrahydrocannabinol (THC), opiates (6-monoacetylmorphine, morphine, codeine, hydromorphone, hydrocodone, oxycodone, noroxycodone), tramadol, methadone, buprenorphine, fentanyl, ketamine and their respective metabolites, and γ-hydroxybutyric acid (GHB) (Stove et al., 2012).

DBS is repeatedly cited as a simple and rather inexpensive sample collection procedure in which the filter paper can retain many blood components, allowing an easier and even automated blood extraction procedure (Déglon et al., 2012; Versace et al., 2013). DBS can solve several limitations involved in routine blood analysis that concern the intrusiveness of intravenous blood sampling, the long-term analytes' stability in blood, and the complex and time-consuming sample preparation and handling. On the other hand, the limitations of DBS sampling include the problems posed by the hematocrit effect, the difficulty to perform repeated analysis due to the small blood volume collected, and the referability of the results obtained with DBS to the conventional cut-off values and normal ranges defined in whole blood analysis (Jantos & Skopp, 2011).

The analysis of target analytes after DBS collection may be performed by several analytical techniques, according to the application: immunoassays, gas chromatography (GC), liquid chromatography (LC) coupled to ultraviolet (UV) or fluorescence detectors, to mass spectrometry (MS) or tandem mass spectrometry (MS/MS). Due to the technological advances over the last decade, LC-MS/MS instruments currently achieve the highest levels of sensitivity and specificity in targeted drug analysis. Extreme sensitivity is obtained by triple quadrupole detectors operating in the multiple reaction monitoring mode (MRM), while the newest TOF (time-of-flight) and Orbitrap analyzers additionally allows high-resolution mass analysis and accurate determination of elemental composition, resulting in accurate qualitative and quantitative analysis of substances present in blood even at extremely low concentration (Antelo-Domínguez et al., 2013; Joye et al., 2019; Kacargil et al., 2020; Keevil, 2011; Li & Tse, 2010; Sadler Simões et al., 2018; Stove et al., 2012). The present review provides an overview of the most recent studies that propose easy and highly sensitive procedures for the detection of drugs of abuse after DBS sampling.

Detection of drugs of abuse by DBS: recent advances

Advantages of DBS

The greatest assets of DBS are the ease of sampling and the fast sample handling, both being crucial aspects in forensic toxicology. For example, rapid sampling is essential in cases of drug-facilitated sexual assault (DFSA) and of driving under the influence of drugs (DUID). In both cases, the sample needs to be collected as soon as possible, since the target substances are generally metabolized in a few hours rapidly becoming undetectable in the primary matrix. On the other hand, alternative matrices such as oral fluid and urine suffer from drawbacks, including controversy about the convertibility between the detected drug concentration and the original dosage and their proneness to alteration (mouth washing or urine exchange) (Huestis et al., 2011). DBS provides reliable estimation of blood concentration without requiring dedicated personnel and a medical office for blood collection.

Typical forensic casework involves extraction of blood volumes ranging from 100–2000 µl depending on the chosen procedure (liquid-liquid extraction or solid-phase extraction) and instrumental analysis (GC/MS or LC-MS/MS), while DBS needs only 10-100 µl of blood sample which can be collected by just pricking a finger or a heel (Watterson, 2015).

Another important aspect of the use of DBS for blood sampling is the improved stability of the contained drugs after being spotted and dried, because of the blood immobilization within the paper matrix (Alfazil A. A. & Anderson R. A., 2008; Garcia Boy et al., 2008). DBS substrates consist of paper chemically treated to inhibit bacterial growth and enzyme activity that would possibly drive analytes' degradation. As a matter of fact, most drugs have shown better stability when dried onto a filter paper than in whole blood, which represents a key issue when time elapses between sampling and analysis, and transportation or storage are needed before the sample can be processed (Cone, 1995; Rook et al., 2006). This is particularly crucial for some drugs of abuse, such as cocaine, heroin, and GHB, that have short a half-life time and limited stability. In particular, cocaine intake can be confirmed by identifying its main metabolite, benzoylecgonine (BZE), whereas heroin abuse cannot be simply deduced from the presence of its main metabolite and hydrolysis product, morphine, because the latter may also arise from a licit pharmaceutical drug, codeine. The case of GHB, also produced by endogenous biochemical processes, is even more complex, since its abuse or unaware administration is ascertained by measuring its excess in blood with respect to the physiological level, which rapidly levels off either by metabolism or degradation

(Stove et al., 2012). For these reasons, both drugs and their intake-suggestive metabolites need to be stabilized. For example, stabilization of cocaine and of 6-acetylmorphine (the first product of heroin metabolism) under DBS conditions has been demonstrated (Sadler Simões et al., 2018).

DBS is also offered for checking drug abuse epidemiology in nightlife environments, resulting in a powerful tool for large-scale investigation of the diffusion, trends, addiction rates, and other physical and social effects of drugs intake and abuse together with statistical analysis, provided that individual consent is mandatory (Sañudo et al., 2015; Wood et al., 2009).

Another demonstrated application of DBS is the follow-up of drug and alcohol addicts' treatment. DBS is used to control abstinence from drugs and/or intake of substitution medication offering an alternative to routine urine testing in which no risk of sample falsification/alteration is possible (Stove et al., 2012). Obviously, DBS involves minimal patient discomfort, unlike intravenous blood sampling, making it admissible on a routine basis. Moreover, DBS involves minimal sample handling which is also an important feature to protect the nursing personnel from the risk of HIV infection, hepatitis or other blood viruses, more frequently affecting drug addicts (Brambilla et al., 2003; Mei et al., 2001).

As far as newborn screening is concerned, DBS has been proposed for studying the prevalence of cocaine and tobacco products intake among pregnant women near childbirth, via the analytical determination of their respective metabolites, benzoylecgonine and cotinine (Stove et al., 2012).

Limitations of DBS

Although DBS solves all problems associated with venipuncture, including also excessive blood volume sampling, the opposite problem combined with the need of controlling the blood distribution homogeneity in the spot emerges from DBS collection. In particular, it is not obvious that the blood deposition onto the card is adequate and uniform. Older studies have tested DBS using around 100 µl (Garcia Boy et al., 2008; Jantos & Skopp, 2011) even if this volume corresponds to intentionally spotted samples after intravenous collection. As observed from recent applications to real samples, the blood volume obtained by a finger prick and directly deposited onto the card does not exceed 30-40 µl. Moreover, the area where the analyte expands to is

possibly smaller than the spot itself, drawing attention on the correct procedures for spotting the card, estimating the collected volume, and recovering the blood spot.

These variables are connected to other blood parameters that should be considered, for example the hematocrit effect, that mainly affects the deposition area and the analytes distribution in it. A clarifying study has been published from *Veghle et al.* (Velghe et al., 2019) concerning the hematocrit effect and the manageable practices that can be used to circumvent the inherent analytical problems. Notably, the hematocrit bias can be overcome either by using controlled and fixed volumetric application of blood or by correcting it with commercially available automated DBS analyzers.

Further risks arise from manual cutting/punching the spot, that are avoided when an automated DBS extractor is used. In the manual procedure, the paper undergoes several treatment steps (cutting, transfer between tubes, extraction with solvents, centrifugation) which require skilled operators not to makes the whole procedure prone to avoidable mistakes. For example, it is important to punch the correct DBS area, in order not to reduce the available material and consequently the sampled volumes, the analytical results, and their comparability.

Lastly, the application of DBS method to any sort of forensic cases (drug monitoring, workplace drug-testing, newborn screening, *etc.*) consistently requires instrumental methods provided with high sensitivity, to support conclusions with unequivocal data, and bearing in mind the limited amount of analyte present on the card and typically arising from less than 30 µl blood sampling.

Recently proposed methods and applications

In the last few years, significant laboratory development in DBS preparation, extraction, and results acquisition has been observed. For the data acquisition stage, hyphenation of UHPLC with either MS/MS or HRMS proved to provide adequate sensitivity to detect analytes at trace level in the tiny amount of blood sampled by DBS. The most persuasive studies are those that include real samples analysis and compare the results obtained after DBS sampling with the ones arising from traditional routine methods. The desirable final goal of these studies is actually to prove that DBS-based methods are suitable for the detection of the most common drugs of abuse and their metabolites under standard routine conditions and provide results similar to

conventional analysis so as to be complementary to it whenever DBS is preferable to intravenous sampling.

Sadler Simões et. al (Sadler Simões et al., 2018) developed and validated an easy and quick method for the analysis of 11 illicit drugs of abuse based on DBS sampling and using UPLC-MS/MS for the instrumental determination. The preparation is simple: 50 µl of whole blood are spotted on a DBS card, allowed to dry off; then, 3 ml of methanol/acetonitrile (3:1 v/v) and 25 µl of deuterated internal standards (ISTD) are added as an extraction solution. After stirring, sonication and centrifugation, the solvent was dried under a nitrogen flow at 35ºC. The residue was reconstituted with 100 µl mobile phase and 10 µl were injected into the UPLC-MS/MS system. The method includes validation of several parameters, including LOD, LOQ, matrix effect and recovery, accuracy, and precision. The results show LOD and LOQ values at 0.5-5 ng/ml and 1-5 ng/ml for all analytes. In the discussion section, this study offers a detailed comparison of LOD and LOQ values for drugs of abuse, obtained in previous studies that followed similar approaches but used slightly different experimental parameters (Alfazil A. A. & Anderson R. A., 2008; Ambach et al., 2013; Clavijo et al., 2011; Garcia Boy et al., 2008; Jantos R. et al., n.d.; Langel K. et al., 2011; Lauer E. et al., 2011; Sosnoff et al., 1996; Thomas et al., 2012; Versace et al., 2013). The method used is similar to the one proposed by *Odoardi et al.* (Odoardi et al., 2014), which demonstrated the detection of drugs of abuse and their metabolites with DBS and subsequent UHPLC-MS/MS analysis obtaining LODs at the range of 0.05–1 ng/ml. Moreover, this work tests and demonstrates the stability of the analytes into different storage temperatures (-10ºC, 2-8ºC and room temperature) for 8 months and compares these results with those reported in previous literature focused on 6-AM, MDMA, amphetamine, cocaine, and their metabolites. Finally, the new method was applied to 64 real samples and the results were compared to those from validated routine whole blood analysis using linear regression analysis (paired t-test and the Bland-Altman statistical method) from which no significant differences were revealed.

In a similar context, *Kacargil et al.* (Kacargil et al., 2020) presented the development, validation, and application to real samples of a DBS method based on LC-MS/MS and aimed at the detection of illicit drugs. This approach used higher volumes of spotted blood (100 µl) and extraction solvent (4 ml), and involved higher extraction time and longer instrumental analysis than that previously cited. Method validation and stability tests were accomplished similarly. From real sample testing, a

significant difference between the two approaches is that in *Sadler Simões et al*. paper, morphine yielded the lowest extraction efficiency, while in *Kacargil et al.* work morphine provided the best results, even if the Authors themselves admitted the possibility that degradation may have produced some biases in their data. For example, only in 3 out of 13 real samples, which had previously tested positive for codeine, were confirmed by DBS testing. Codeine degradation may in turn increase the morphine positivity rate. In general, it is confirmed that the original drugs stability is increased when blood is spotted on a DBS card and allowed to dry.

Chepyala et al. (Chepyala et al., 2017) developed an ultra-high-performance liquid chromatography - ion booster - quadrupole time-of-flight mass spectrometry (UHPLC-IB-QTOF-MS) method for the analysis of 57 compounds recovered from DBS cards and including the most abused drugs, namely amphetamines, opioids, cocaine, benzodiazepines, barbiturates, and other emerging drugs of abuse and their metabolites. 25 µl of spiked whole blood was spotted on the filter paper and dried. The dried blood spot was extracted with 200 µl 80%/20% acetonitrile/water mixture and centrifuged. 170 µl of the supernatant was evaporated under nitrogen flow and finally reconstituted with 150 µl of deionized water, vortexed, filtered, and injected into the LC system. The ion booster (IB) source was used to improve the detection sensitivity. Based on the ESI principles, the IB source comprises a heated vaporizer, a charge-HV transfer tube, and the additional use of nitrogen as a sheath gas to improve the mobile-phase evaporation and the analytes' ionization. After optimization of the experimental parameters (including ion polarity) for all the analytes, improvements ranging from 1.5 to 14-fold with respect to conventional ESI was observed, allowing the detection of 95% of the target analytes below the respective therapeutic intervals. Such an improvement makes the method particularly suitable for toxic and forensic drug screening.

Recently, *Joye et. al* (Joye et al., 2019) developed a LC-HRMS procedure for the screening of drugs of abuse after DBS sampling. Three main differences with respect to previous methods were proposed: i) the target analytes panel was expanded by adding new anticonvulsants and THC-COOH to the common drugs of abuse, ii) the extraction method was diversified, and iii) the mass detection was based on high-resolution data. The extraction was performed on 2 spots of 10 µl each: the first was extracted with 100 µl methanol while the second with 100 µl borate buffer 0.5 M at pH 9.5 and 300 µl dichloromethane/hexane/ethyl acetate mixture (5:4:1). The two-

extraction solution were mixed, then the solvent was evaporated, the residue was solubilized into 50 µl water, and injected into the LC-HRMS instrument. Validation of the method was performed on the target analytes (for example, LODs and limits of identification (LOIs) were calculated for 30 substances), but the full-scan MS data acquisition allowed by Orbitrap technology enables the qualitative detection of more than one thousand drugs. This is particularly important for new psychoactive substances (NPS) which are normally not tested in the targeted methods addressed to conventional drugs but are increasingly abused to circumvent testing and to evade national and international legislation. The possibility of large-scale screening for all the reported classes of drugs was confirmed by testing 104 real samples and comparing the present method to other routinely applied methods.

The use of DBS for the detection of drugs of abuse is also applicable when postmortem samples are available, particularly when small blood volume is available. *Moretti et al.* (Moretti et al., 2018) developed and validated a LC-MS/MS method for the identification of cocaine and its metabolites in DBS using postmortem samples. 45 real postmortem cases were evaluated during the period of one year and the results were compared to those arising from routine whole blood analysis showing good qualitative and quantitative correlation. Few years later, *Moretti et al.* (Moretti et al., 2021) developed a study to compare the behavior of two different paper substrates for DBS sampling used in qualitative and quantitative analysis of postmortem cardiac blood specimen. Twenty different cases were examined, involving antidepressants, antipsychotics, benzodiazepines, and their metabolites, from which it was concluded that both types of filter papers were equally applicable, whereas specific attention have to be paid to the type of extraction solvent used, possibly resulting in different extraction yields from the two cards for a few analytes.

An innovative proposal that solves the main limitations of DBS sampling is the fully automated DBS method that couples a DBS autosampler with an LC-MS/MS system. *Luginbühl and Gaugler* (Luginbühl & Gaugler, 2020) presented step by step the workflow of such a proposal. The DBS autosampler is a system in charge of all the steps preceding injection: from the sample information recovery and quality control check to internal standard application and spot extraction. At the end, a start signal triggers the sample injection and instrumental run from the autosampler to the LC-MS/MS system. Specifically, *Gaugler et al.* (Gaugler et al., 2018) reported a fully automated screening for 12 drugs of abuse with DBS and online LC-MS/MS analysis.

A stock standards solution was mixed with the donor blood in four different concentrations to prepare the calibration curve. 15 µL aliquots were spotted onto the card, dried at room temperature, and extracted into a 20 µL sample loop with a mixture of methanol and water (70:30, v/v) and then injected. Another forensic study from the same Authors (Gaugler et al., 2019) was focused on routine DBS screening in workplace testing, and included 28 compounds. The method robustness was confirmed on real blood samples. The major advantages of the automatic procedure is that the direct sample elution from the DBS card eliminates the need of several consumables, such as sampling tubes, pipette tips, and glass vials and exclude the chance of errors due to human sample handling. These advantages, combined with high performance MS instrumentation (either HRMS or MS/MS) is likely to make the DBS method fast, efficient, and reliable.

Conclusions

The methods of toxicological analysis applied on samples collected by DBS have already gone far beyond the stage of proof-of-concept and are earning a chance to progressively substitute for traditional methods carried out on whole blood from intravenous sampling. The DBS methods have been validated for quantitative determinations, their results have been cross-checked with respect to conventional methods, and successfully applied to real samples. Moreover, improved sample and analyte stability had been demonstrated on DBS cards. Still, DBS collects a very limited amount of blood and produces spatially inhomogeneous samples. Therefore, DBS methods for toxicological analysis are required to go through large-scale trials on real samples to standardize further the experimental conditions and normalize the possible limitations that these methods may pose. Also, wider “on-field” sampling campaigns (e.g., road-side testing, drug testing in nightclubs) are needed to definitely prove the routine applicability of DBS sampling in contexts where traditional methods of blood sampling are totally impracticable. In parallel, DBS practices and results should be compared with those using different alternative matrices in “on-field” controls (for example, oral fluid in road-side testing, hair or urine in drug withdrawal controls) to verify the respective advantages and limitations and choose the most effective approach for each circumstance.

Detection of drugs of abuse by DBS: recent advances

Liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) still represents the preferred instrumental method among those commonly available in forensic laboratories, because of its inherent high speed, sensitivity, accuracy, and precision, especially in targeted operating mode (selected reaction monitoring - SRM). In alternative, LC-HRMS offers an equally selective detection mode but frequently combined with the availability of full-scan data, of unique usefulness whenever postponed reevaluation of the analytes' panel becomes necessary. This is an increasingly common scenario in the rapidly evolving market of drugs of abuse and novel psychoactive substances, that are habitually not included in the targeted SRM methods of analysis but have to be prospectively integrated into the forthcoming wide screening procedures.

The HRMS offers the ability to perform both qualitative and quantitative analysis and this opportunity is appealing for forensic perspective as the information obtained is not only about the target analyte but also for its metabolites and potential biomarkers of interest. The greater mass range of HRMS in comparison to triple-quadrupole instruments offers the possibility of analyzing large molecules, for both identification and direct quantification. HRMS provides highly specific characterization of the analytes, reducing or eliminating the interferences that occasionally affect the determinations made by triple quadrupole instruments. Moreover, the use of electrospray ionization (ESI) or ion booster (IB) source can further improve the MS sensitivity for a wide range of drug candidates and metabolites, as *Chepyala et al.* proposed. As demonstrated by *Joye et al.*, more than satisfactory results were obtained when a quadrupole-orbitrap HRMS operating in DDA full-scan approach was used. Indeed, 99% of the DBS cases were identified by the quadrupole-orbitrap HRMS, whereas at concentrations between 0 and 10 ng/ml 40-50% of the substances detected by the HRMS were not identified by LC-MS/MS. Remarkably, broad-spectrum HRMS screening methods can become of particular interest owing to the limitations to targeted LC-MS/MS methods presented by the progressive introduction of new drugs of abuse in the illegal market. The triple quadrupole tandem mass spectrometer will likely remain the preferred instrument for quantitative detection of traditional drugs of abuse, usually predetermined as target analytes. However, the retrospective investigation represents an added value for investigation of DBS samples, especially when the small amount allows only one analysis, and especially in forensic

labs where there is a greater need to maximize the range of detectable compounds (Massano et al., 2022).

Several forensic laboratories are currently increasing their interest in DBS and consider it the potential procedure of choice for several reasons. DBS guarantees an unquestionable ease of sampling, as the collection of capillary blood is minimally invasive and there is no need for trained medical staff to perform its sampling. DBS also assures fast result turnaround, and early stabilization effects on drugs by inhibiting their degradation. The whole procedure also complies with the aims of Green Analytical Chemistry and Sustainable development (*The Sustainable Development Goals Report*, 2022), that nowadays represents a must, as it involves a small sample volume and consequently demands a lower amount of extraction solvent and less energy consumption, both leading to lower waste production and environmental impact. Likewise, DBS cards shipping is significantly simplified, requiring no special conditions and reduced storage space, making the technique applicable for testing and research in every part of the world, also under uncomfortable conditions. In conclusion, it is predictable that DBS will play a rapidly increasing role in the toxicological testing activity toward illicit substances including old and new drugs and doping agents, so as to expand the feasibility of blood testing and effectively tackle the proliferating problem of illicit substances abuse.

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Table 1: Summary table of the main parameters reported by each study

ANALYTES	FILTER PAPER	SAMPLE VOLUME (µl)	EXTRACTION SOLVENTS	TECHNIQUE	EQUIPMENT	RUN TIME (min)	LOD (ng/ml)	STABILITY TEST	REFERENCES
Amphetamine,6-AM, Benzoylecgonine, Cocaine, Codeine, EDDP, MDA, MDMA, Methadone, Methamphetamine and Morphine	Whatman BFC 180 bloodstain cards	50	Methanol/acetonitrile	UPLC-MS/MS	Waters Acquity UPLC coupled with Waters TQ Detector, Waters Acquity UPLC® HSS T3 column (100 × 2.1 mm, 1.8 µm)	10	0.5-1	RT, 2-8°C and -10°C for 8 months	Sadler Simões *et al.* (Sadler Simões et al., 2018)
Amphetamine, Methamphetamine, MDMA, MDA, Morphine, Codeine, Cocaine, Benzoylecgonine and Methylecgonine	Sartorius Stedim Biotech Sample Carrier Paper	100	Ethylacetate/methanol	LC-MS/MS	Shimadzu CBM-20A UFLC* coupled with Shimadzu 8040 Mass Spectrometry, Allure® Pentafluorophenylpropyl (PFPP) column (50 mm × 2.1 mm, 5 µm)	20	0.1-6.52	RT, 4°C, -20°C for 15, 35 and 95 days	Kacargil *et al.* (Kacargil et al., 2020)
Amphetamines, opioids, cocaine, benzodiazepines, barbiturates, and metabolites (total of 57 compounds)	Whatman 903 card	25	ACN 80%	UHPLC-IB-QTOF-MS	Agilent 1290 UHPLC system coupled with a Bruker maXis QTOF with IB source, Agilent Poroshell EC-C18 column (2.1 × 100 mm, 2.7 µm)	20	0.2-35.7	RT, -80°C for 1 and 6 months	Chepyala *et al.* (Chepyala et al., 2017)
Amphetamines, benzodiazepines, cocaine, antidepressants, neuroleptics,	Whatman Protein saver DBS	10 × 2 (2 spots)	Methanol, Borate buffer 0.5 M pH 9.5 and DCM: Hexane: Ethyl Acetate (5:4:1) (2 spots extraction)	LC-HRMS	Thermo Scientific LC-Q Exactive Plus system, Phenomenex 2.6 mm C18 column (2.1 × 50 mm)	0	<1-10	no	Joye *et al.* (Joye et al., 2019)

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opioids, NPS, anticonvulsants and THC-COOH (total of 30 compounds)									
Alprazolam, Amphetamine, Cocaine, Codeine, Diazepam, Fentanyl, LSD, MDMA, Methadone, Methamphetamine, Morphine and Oxycodone	Ahlstrom TFN filter paper CAMAG	15	Methanol/water (70:30 v/v)	LC-MS/MS	CAMAG DBS-MS 500 system, Shimadzu UHPLC system coupled with 8060 triple quadrupole, Shim-pack GIST column (2.3 x 50 mm, 5 µm C18, PN 227-30017-3)	10	0.05-30	no	Gaugler *et al.* (Gaugler et al., 2018)
Amphetamines, benzodiazepines, cocaine, heroin, cannabinoids (total of 28 compounds)	Ahlstrom TFN filter paper	20	Methanol/water (70:30 v/v)	LC-MS/MS	CAMAG DBS-MS 500 system, Shimadzu UHPLC system coupled with 8060 triple quadrupole, Shim-pack GIST (2.1 × 50 mm, 2 µm C18) and Kinetex (2.1 × 100 mm, 2.6µm, XB-C18) columns	5	10-100	no	Gaugler *et al.* (Gaugler et al., 2019)