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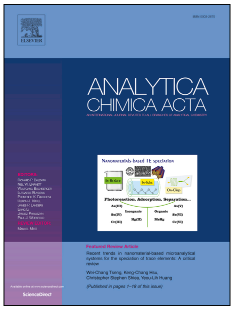

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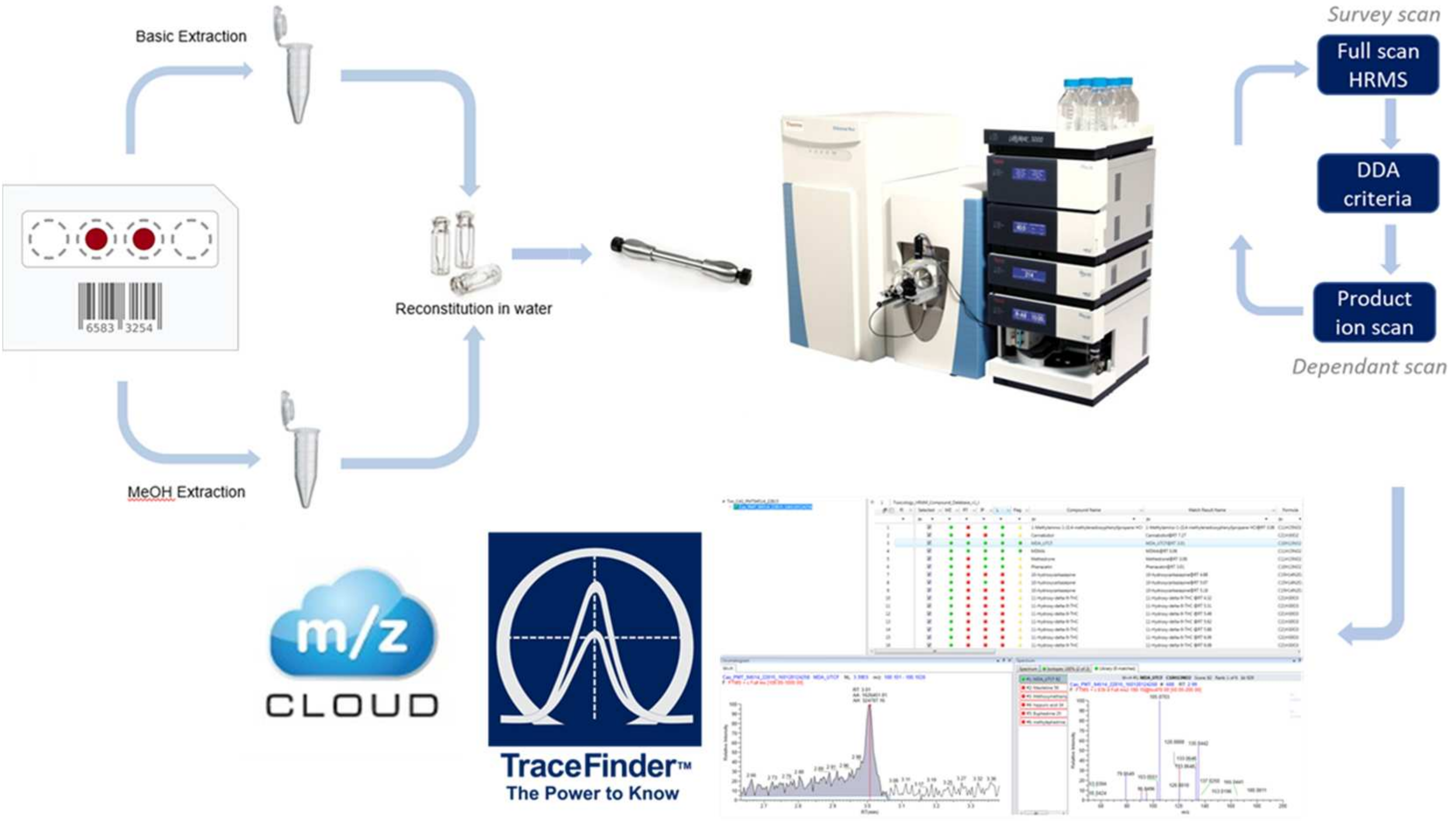
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Liquid Chromatography –High Resolution MS-based assay for broad-spectrum drug screening of dried blood spot as microsampling procedure

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Nonstandard abbreviations: STA, systematic toxicological analysis; GUS, general unknown screening; LC, liquid chromatography; HRMS, high resolution mass spectrometry; DDA, data-dependent acquisition; DBS, dried blood spot; DMS, dried matrix spot; MRM, multiple reaction monitoring; LOI, limit of identification; LOD, limit of detection; DIA, data-independent acquisition; SWATH, sequential window acquisition of all theoretical fragment-ion spectra; MSX, multiplexed.

Abstract

Background: Hyphenation of liquid chromatography (LC) with high-resolution mass spectrometry (HRMS) offers the potential to develop broad-spectrum screening procedures from low volumes of biological matrices. In parallel, dried blood spot (DBS) has become a valuable tool in the bioanalysis landscape to overcome conventional blood collection issues. Herein, we demonstrated the applicability of DBS as micro-sampling procedure for broad-spectrum toxicological screening.

Methods: A method was developed on a HRMS system in data dependant acquisition (DDA) mode using an extensive inclusion list to promote collection of relevant data. 104 real toxicology cases were analysed, and the results were cross-validated with one published and one commercial screening procedures. Quantitative MRM analyses on a triple quadrupole instrument were also performed on identified substances as a complementary confirmation procedure.

Results: The method showed limits of identification (LOIs) in appropriateness with therapeutic ranges for all the classes of interest. Applying the three screening approaches on 104 real cases, 271 identifications were performed including 14 and 6 classes of prescribed and illicit drugs, respectively. Among the detected substances, 23% were only detected by the proposed method. Based on confirmatory analyses, we demonstrated that the use of blood micro-samples did not impair the sensitivity allowing more identifications in the low concentration ranges.

Conclusion: A LC-HRMS assay was successfully developed for toxicological screening of blood microsamples demonstrating a high identification power at low concentration ranges. The validation procedure and the analysis of real cases demonstrated the potential of this assay by supplementing screening approaches of reference.

1. Introduction

Systematic toxicological analysis (STA) is of primary importance in both clinical and forensic toxicology. It generally consists of a combination of analytical strategies including immunoassays, and chromatography hyphenated to mass spectrometry [1-3]. In STA, orientation tests and preliminary screening are performed, which can be referred as general unknown screening (GUS)[4]. The goal of this procedure is to conclude in a precise and unambiguous way if any substance of toxicological interest has been consumed or not prior confirmatory quantitative analysis[5, 6]. This challenging task depends on the biological matrix, sample preparation, analytical technique, and the compound database.

Currently, liquid chromatography (LC) hyphenated with mass-spectrometry (MS) is partially replacing gas chromatography (GC)-MS approaches regarding GUS in blood and urine and numerous applications have been mentioned[6, 7].

The introduction of high-resolution MS (HRMS) analyser and especially Orbitrap technology, gives the opportunity to investigate several analytical strategies including full scan data dependent acquisition (DDA) [8-10]. The latest generation of Fourier transform MS, routinely reaches mass resolution above 70,000 at 1-ppm mass accuracy and high spectral resolution capacities. Continual improvement of scan rate frequencies and dynamic range lead to instruments particularly adapted for large-scale comprehensive screening in complex matrices [11-13]. The increased mass accuracy allows to facilitate identification by reducing the number of possible chemical formulas [6]. Lastly, this technology offers fast positive/negative polarity switching at high scan rates allowing the simultaneous analysis of a wide range of substances [14]. Therefore, HR full-scan methods are very suitable for the development of large-scale screening procedure and especially for drug screening [3, 15]. By improving the mass resolution power, HRMS increases the selectivity, therefore reducing the potential interferences [10]. The associated improvement in terms of sensitivity could allow to reduce the volume of biological matrices used for the screening procedure.

Currently, urine is the gold standard regarding screening approaches since its sampling is simple and non-invasive. Moreover, urine is relatively poor in proteins and lipids that could interfere with the signal of the analytes and concentrate most analytes reducing potential sensitivity issues [16]. Compared to urine, blood sampling presents significant advantages since it is difficult to counterfeit, and the toxicological interpretation of concentration is facilitated making it the gold standard for confirmatory analysis [17]. However, blood sampling induces several logistical and analytical issues. Indeed, classical venepuncture is invasive, requires special logistic and medical supervision [18]. In addition, in some situations, especially concerning problematic and vulnerable patient population, only limited volumes of blood are available [19]. To bypass those issues, the use of cellulose paper cards has been mentioned [20]. The first use of dried blood spot (DBS) has been reported by Guthrie and Susie more than 50 years ago, for paediatric purpose [21]. Among advantages, DBS sampling requires the collection of a small volume of blood (5-10 µL). Moreover, it is performed by finger or heel pricking that can be performed by a technician or by the patient himself after minimal training in a non-hospital environment [22]. The adsorption and drying of blood on a solid phase makes analytes less reactive, leading to facilitated shipment and storage and reducing the costs [23]. Lastly, during blood adsorption and drying most pathogenic agents are deactivated leading to a safer handling of samples [24].

Various applications of detection of drugs using dried matrix spots (DMS) [14, 27] and especially DBS sampling have already been presented [7, 23, 28, 29]. For instance, analyses on DBS have been used for both quantitative (quantification of benzodiazepines) [22] and screening approaches either for doping screening [7] or for toxicological analyses using a targeted strategy [30]. Indeed, DBS presents several advantages for MS-based analyses since samples preparation can be accelerated and facilitated. Moreover, using organic solvents, lipids and proteins are mostly being retained on the paper-card allowing the reduction of matrix effects, making this sampling support particularly adapted to MS-based strategies [22, 31-33]. We previously demonstrated the potential of DBS for targeted drug screening using a multiple reaction monitoring (MRM)-DDA approach [30]. In the present study, we extend the screening capabilities from DBS samples using the analytical advantages brought by the HRMS, notably by broadening the number of possible identifications. In this way, a full-scan-DDA LC-HRMS method was developed using an inclusion list of more than 1000 compounds including all classes of interest. The developed assay requires limited sample preparation and allows the identification of a wide-range of compounds. Representative substances were tested for the chromatographic and MS parameters optimisation including non-exhaustively amphetamines, benzodiazepines, cocaine, antidepressants, neuroleptics, opioids, NPS, anticonvulsants, and THC-COOH. In a second time, a subset of 30 substances was used for the determination of the limits of identification (LOIs). This method was then assessed using 104 behavioural toxicology real cases. The results were cross-validated by two published screening methods used in routine [30, 34]. Confirmation assessment were also performed using quantitative analysis as a complementary approach to evaluate the efficiency of the developed routine screening method presented herein.

2. Methods

2.1 Standards and reagents

Water, methanol, formic acid (FA), dichloromethane, hydrochloric acid, hexane and ethyl acetate UPLC, potassium and sodium chloride, boric acid, and ammonium formate were purchased from Biosolve, Sigma-Aldrich or Merck. Drug standards were purchased at 1 mg/ml either from Cerilliant or Lipomed. Blank, lyophilized whole blood for method development was purchased from ACQ Science. Protein saver cards for DBS sampling were purchased from Whatman. Whole blood external quality control (EQC) PM100 for pain management and drug of abuse (DoA-I VB low) were purchased from UTAK® (Supplemental Table 1) and Medidrug® (Supplemental Table 2) respectively.

2.2 Sample preparation

For method development, blank whole blood was spiked with drugs at different concentrations (1, 5, 10, 20 and 50 ng mL^{-1}). Methanolic standard were evaporated under a nitrogen flow at room temperature before reconstitution in blood. Then 10 µL were deposited on a DBS filter paper card. Drugs were tested for limits of identification and detection determination, chromatography and identification optimisation including benzodiazepines, NPS, neuroleptics, opioids, antidepressants, synthetic cannabinoids, amphetamines, anticonvulsant, cocaine and cannabinoids. A double extraction process on DBS was developed. One spot was extracted using 100 µL of methanol and mixed for 2 minutes. A second spot was extracted using 100 µL of borate buffer 0.5 M pH 9.5, after mixing for 2 minutes, 300 µL of DCM: Hexane: Ethyl Acetate (5:4:1) were added. This second spot was then centrifugated and the organic phase was mixed with the 100 µL of methanol extract from the first extraction in a new tube. Organic solvents were then evaporated at room temperature using a nitrogen flow, and samples were reconstituted with 50 µL of water.

2.3 LC-HRMS method

All samples were injected (10 µL injection volume) using partial loop injection mode on the LC-Q Exactive Plus system (Thermo Scientific, Bremen, Germany). Chromatographic separation using a Thermo Scientific Ultimate 3000 LC system with a Phenomenex 2.6 µm C18 (2.1 X 50 mm) maintained at 45°C. Mobile phase A consisted of ammonium formate 10 mM pH 3.3 and mobile phase B of MeOH with 0.1 % FA. Phase B was ramped linearly from 2 to 98% over 6 minutes. The column was washed at 98 % of B for 3 minutes, followed by a 3.5 minutes reequilibration at 2 % of B. The LC was coupled to the MS via a heated ESI source associated with a Q Exactive Plus operating in full-scan DDMS^2 positive polarity with a Tsim DDMS^2 negative polarity switching between min 6 and 7 only for the specific detection of THC-COOH (supplemental Figure 1A). The ionisation spray voltage was set to 3 kV, sheath gas flowrate was set to 40 and auxiliary gas flowrate to 10 (both in arbitrary unit). HCD fragmentation was performed with an inclusion list containing 1008 compounds using NCE at 70 eV in positive polarity and 30 eV in negative polarity. No dynamic exclusion was set. Resolution was set to 70’000 for the full scan experiment in positive polarity while it was set to 17’500 during the polarity switching and the fragmentation experiments.

2.3 Data analysis

Data analysis was performed using TraceFinder (Thermo Scientific) and a database containing more than 1000 compounds (Supplemental Table 3). Four criteria were used for substance identification including the mass over charge ration (m/z), the isotopic pattern (IP), the retention time (RT) and the library search (LS) based on the fragmentation spectra comparison. Peak detection was based on a S/N threshold of 10 with a 10 ppm tolerance. Library search was considered successful with a match greater or equal to 50 %. IP needed to fit over 65% allowing 10 ppm mass and 20% intensity deviations and RT was considered with a 30 seconds precision. Substances identification was still possible if either the RT or the LS do not match..

2.4 Method evaluation

LOIs were measured using a list of 30 substances including most classes of interest (Table 1). LOIs were evaluated by injecting 5 spiked replicates at 1, 5, 10 and 20 ng mL^{-1}. To minimise the risk of false negative and as described elsewhere, LOI was considered as the lowest concentration at which the substances are detected and identified in all five replicates based on the four criteria previously described [35, 36]. Limits of detection (LODs) were defined as the minimal concentration at which at least the accurate mass precursor ion is detectable [14]. Method reproducibility and instrument response were then evaluated using whole blood controls EQC UTAK® pain management PM 100 (Supplemental Table 1) and the lowest concentration Medidrug® for drug of abuse (Supplemental Table 2) spotted on a filter paper card. Each QC was injected at the beginning and the end of each sequence. A QC was considered acceptable when all its substances targeted by the method were identified regarding the four criteria. Selectivity was assessed by injecting ten different blood samples spotted on DBS. Matrix effect (ME) and recovery (RE) was evaluated according to the approach described by Matuszewski et al [37], three sample sets were prepared including 30 representative substances (Table 1). Five different blank blood sample or neat standard were spiked with those substances at two concentration levels (20 and 200 ng mL^{-1}). Sample set 1 represented neat standards. Sample set 2 blank blood matrix spots spiked after extraction while sample set 3 represented blank blood matrix spiked and spotted before extraction.

To evaluate and confirm the efficiency of the whole process, 104 samples from forensic and clinical toxicology were analysed. Those samples were also analysed by two other routine LC-MS methods. The first one was developed for forensic toxicology using one 10 µL DBS sample. The spot was introduced into a vial with 100 µL of methanol and injected on the LC-MS/MS system. The analysis was performed on a Qtrap® 5500 (AB, Sciex) working in targeted multiple reaction monitoring (MRM)-DDA. The chromatography was performed both on a reverse phase (RP) and a Hilic column in parallel [30]. The second one was developed for clinical toxicology on a Toxtyper LC-IT-MS System (Brucker). Sample preparation was based on alkaline liquid/liquid extraction with sodium carbonate 1M and butyl acetate from 500 µL of whole blood. The method was based on low-resolution ion trap technology after a standard RP chromatographic separation. The MS experiment was performed in full-scan DDA with continuous positive/negative ionisation mode switching [34]. Identifications performed by the 3 methods have been compared as a cross-validation step. The detection of a substance was confirmed either if it was detected by at least two of the assessed methods or if its identification was unambiguous. Carryover was evaluated by injecting a methanol sample after each analysed blood sample and by analysing 10 different blank whole blood samples after the injection of the 104 real cases. In addition, sensitivity assessment and identification confirmation has been processed using quantitative analysis.

3. Results and discussion

3.1 Sample preparation

DBS provides several well-known advantages toward sample collection. Moreover, the use of blood micro-sampling was described as a promising technique to simplify sample preparation prior to the analysis and reduce the solvent volumes [30, 38]. Using only two 10 µL spots, a limited sample preparation was sufficient to sensitively detect all the classes of drugs of interest in their therapeutic or legal range. The development of such micro-sampling strategies presents an alternative for blood collection in non-hospital environment facilitating potential large cohort studies [25]. In addition, the combination of simplified sampling with large-scale adaptative screening approaches could be a public health asset for monitoring habits of consumption and for targeted prevention. For instance, the emergence of quickly evolving novel psychoactive substances (NPS) emphasize the risk for the population requiring large-scale adaptative analytical strategies [26].

The method was validated according to the recommendations regarding qualitative approaches [39, 40]. Water was selected as reconstitution solvent based on a compromise between specificity for a maximum of compounds with different physico-chemical properties and chromatography. Selectivity was investigated using ten blank blood samples spotted on DBS and ten clean filter paper cards. No effects were observed extracting and analysing clean paper spots, suggesting that no substances were released from the paper during the extraction procedure. No interfering compounds impaired the detection of the substances of interest analysing blank DBS samples. The systematic injection of a blank methanol after every analysed samples did not revealed carry-over even after injection of the highest EQC level (i.e. no remaining peaks were observed in the injected methanol for those exact masses). This was confirmed from real cases analyses since no carryover was observed after the injection of samples containing high concentrations of benzoylecgonine (2900 ng mL^{-1}), tramadol (1400 ng mL^{-1}) or zolpidem (1200 ng mL^{-1}). The RE and ME results for the DBS extraction process are depicted in Table 1. The mean ME at 20 ng mL^{-1} was 34 % ranging from -53 % to 319 % and 13 % at 200 ng mL^{-1} ranging from -42 % to 178 %. RE was ranging from 36 % to 121 % for respectively benzoylecgonine and cocaine at 20 ng mL^{-1} and from 22 to 71 % for respectively methamphetamine and fluoxetine at 200 ng/ml. Maximal ion suppression was observed for THC-COOH while maximal ion enhancement was observed for fluoxetine. Those ME and RE values are in accordance to previous studies [7, 32, 41]

3.2 Detection and identification

Despite several advantages provided by DBS, the use of micro-sampling requires highly sensitive instruments. LODs and LOIs for the 30 model compounds are listed in Table 1. As described above, LOI was defined as the lowest concentration where at least 3 of the 4 identification criteria (m/z, IP, RT and LS) were fulfilled while LOD was based only on the parent peak detection explaining the slight differences between those parameters [11, 14]. LOIs were determined to be equal or lower than 20 ng mL^{-1} for all the 30 substances tested including 11 different classes of molecules, which was the initial goal. LOI was measured at 20 ng mL^{-1} for one substance (Gabapentin), while 4 substances have been successfully identified in all 5 replicates at 5 and 10 ng mL^{-1}. For most of the tested substances (63 %) the LOI was assessed to be 1 ng mL^{-1}. Determined LOIs were confirmed by the identification of all substances in the lowest EQC levels (Supplemental table 1 and 2). LOD and LOI were found to be in adequation with the therapeutic ranges [42] or the legal thresholds according to the Swiss legislation [43]. Regarding identification, parameters were selected to maximise the method efficiency and reduce the potential number of false negatives. Further investigation by the operator is required when either the library spectra or the retention time does not match. The use of such an approach allows identifications by post-processing after database adjustments. By relaxing those identification criteria, the sensitivity is increased leading to a higher risk of false positive identification. Therefore, a careful data handling is performed by the operator to assess whether it is a true or false positive.

3.3 Application of the method on real cases

From the three different screening methods, 271 identifications were validated among the 104 clinical and forensic toxicology blood samples (Supplemental Table 4). In total, six classes of illicit drugs (amphetamines, cannabinoids, opioids, NPS and LSD) and 14 classes of prescribed drugs including non-exhaustively antifungals, antimalarials, antidiabetics, antihistaminic, beta-blockers, or proton-pump inhibitors were detected. The prescribed drugs were summarized as five classes of prescribed drugs (benzodiazepines, neuroleptics, antidepressants, anaesthetics/analgesics and other drugs) for better representation. In 24 cases, no compound was detected and identified while in the 80 other cases, a total of 79 different substances were confirmed. The targeted MRM-DDA approach on DBS allowed the identification of 57 % of the substances while using the Toxtyper® on whole blood this number has increased to 72 %. Using the HRMS-DDA full-scan approach, 99 % of the “hits” were identified on DBS confirming the efficiency of the developed method (Figure 1 A). As expected THC-COOH was the most detected substance being present in more than 46 % of all the cases (104) (Figure 2). Without considering THC-COOH which is targeted only by the HRMS-DDA approach, the percentage of identification respectively increases to 87% and 69 % for the Toxtyper and the MRM-DDA. Cocaine, benzodiazepines and amphetamines were respectively detected in 19 %, 17 % and 8 % of the cases (Figure 2). A total of 18% of the cases were containing either an anaesthetic or an analgesic including ketamine or lidocaine. Other drugs were responsible for 13 % of all identifications while one case of NPS (mephedrone) and one containing LSD were listed. The differences between the percentage of detected substances and the occurrence in cases (reaching more than 170%) can be explained by the concomitant consumption of 2 or more drugs (55 % of the positive cases). Cannabinoids (represented only by THC-COOH) were the most single-drug consumed (80% of the cases positive to only one substance). Quantitative confirmations were performed on qualitatively identified substances targeted by the 3 methods. As confirmed by the quantitative results, the number of identifications using HRMS is increased in the lowest concentration ranges (see Figure 1B). As an illustration, at concentrations between 0 to 10 ng mL^{-1}, 40 % and 50 % of the confirmed substances detected by the HRMS-DDA procedure were respectively not identified by the MRM-DDA and the Toxtyper approaches. At the opposite, the 3 methods were able to detect all the compounds of interest from concentrations higher than 200 ng mL^{-1}. The HRMS improvement in terms of selectivity also results in an increase of sensitivity for full-scan approaches allowing to work with blood micro-samples. Nevertheless, the demonstrated gain of sensitivity also results in higher risks of false positive. Peak area thresholds have been implemented for well-ionised substances and classic contaminants such as cocaine or methadone.

Due to the number of substances to cover and the diversity of physico-chemical properties, the development of an unbiased and sensitive screening procedure is a difficult task [30]. Current HRMS technologies offer the possibility to use either DDA [41] or data independent acquisition (DIA) including sequential window acquisition of all theoretical fragment-ion spectra (SWATH) [44, 45] or multiplexed (MSX) [46]. A recent study demonstrated the interest of SWATH in comparison to conventional DDA for the analysis of coeluting substances in complex matrices [47]. Despite the theoretical advantages of DIA strategies for toxicological screening, these approaches generate complex composite MS/MS spectra, which may hinder the identification of substances in the low concentration ranges. These issues would be overcome with the development of more efficient search algorithms and deconvolution processes for DIA routine applications [48].

Unlike DIA approaches, a limiting factor of DDA using intensity threshold as the main criteria would be the cycle time for fragmentation in case of coelution which can lead to prioritize MS/MS acquisitions of matrix interferences over pertinent compounds [47]. Even if DDA limits retrospective data evaluation because only know precursors are selected for fragmentation, by producing better fragmentation spectra than DIA [47, 49], the use of DDA with a large inclusion list (i.e. more than 1000 substances as demonstrated herein) thus allow the instrument to spend time only on collecting relevant data. Besides, the inclusion list flexibility allows the fast and easy adaptation to any new substances increasing the method identification power.

4. Conclusion

In summary, a large-scale HRMS toxicology screening strategy was developed on DBS samples. The method operating in full-scan DDA using an inclusion list of more than 1000 compounds showed a high identification power in which new substances can easily be implemented. The method was cross-validated with one published and one commercial screening procedures. Confirmation quantitative analyses on identified compounds have demonstrated that despite the use of blood micro-samples the sensitivity was not impaired providing several advantages. Regarding the importance of screening procedures within the STA, rapid and comprehensive GUS tools are necessary and should be proposed. The hyphenation of DBS with HRMS might be an attractive solution combining a friendly sampling process with highly selective and sensitive MS-based detection. This method that was validated [39] and implemented for its use in routine opens notably new toxicological perspectives towards population monitoring in non-hospital environment. In a near future, the development of online shared libraries such as m/z cloud and bioinformatic tools should allow to broaden the number of efficient large-scale screening strategies especially regarding non-targeted approaches.

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Tables:

Table 1: List of the 30 substances used for the method evaluation with their respective limits of identification (LOIs), limits of detection (LODs) and therapeutic ranges or legal thresholds. Matrix effect (ME) and recovery (RE) were evaluated at two concentration levels (20 and 200 ng mL^{-1}) on thoses substances involving a representative panel of the classes of the molecules of interest.

Substance	**LOI (ng mL^{-1})**	**LOD (ng mL^{-1})**	**RE (CV) (20 ng mL^{-1})**	**ME (CV) (20 ng mL^{-1})**	**RE (CV) (200 ng mL^{-1})**	**ME (CV) (200 ng mL^{-1})**	**Therapeutic range/ Legal threshold (ng mL^{-1})**
6-MAM	5	1	90% (6%)	5% (7%)	57% (19%)	-5% (3%)	-
Aminoclonazepam	10	10	72% (10%)	-27% (4%)	41% (10%)	-19% (5%)	-
Amitryptiline	1	< 1	94% (10%)	208% (23%)	46% (20%)	100% (12%)	50-300
Amphetamine	10	1	49% (5%)	-5% (7%)	25% (5%)	48% (4%)	15
Benzoylecgonine	1	< 1	36% (5%)	-16% (5%)	24% (6%)	-8% (2%)	-
Benzylpiperazine	1	< 1	88% (5%)	-3% (8%)	38% (27%)	-16% (5%)	-
Butylone	1	< 1	65% (5%)	-4% (7%)	30% (24%)	-5% (5%)	-
Carbamezapine	1	< 1	89% (4%)	-18% (4%)	55% (10%)	-10% (3%)	200-800
Citalopram	1	< 1	110% (10%)	31% (18%)	61% (17%)	-14% (11%)	50-110
Cocaine	1	< 1	121% (5%)	29% (9%)	60% (11%)	-2% (7%)	15
Codeine	5	1	72% (6%)	-5% (5%)	52% (8%)	-3% (2%)	30-250
Diazepam	1	< 1	88% (8%)	-20% (7%)	47% (7%)	-16% (2%)	100-2000
Fluoxetine	1	< 1	113% (19%)	319% (28%)	71% (19%)	178% (10%)	120-500
Gabapentin	20	5	57% (15%)	-35% (42%)	39% (6%)	-22% (21%)	50-600
Haloperidol	1	< 1	106% (19%)	170% (19%)	57% (19%)	114% (11%)	5-17
Hydroxymidazolam	1	< 1	90% (8%)	-28% (4%)	49% (15%)	-23% (4%)	-
Ketamine	5	1	71% (2%)	6% (7%)	34% (13%)	-3% (3%)	1000-6000
MDMA	1	< 1	68% (3%)	-9% (6%)	33% (34%)	-9% (5%)	15
Methadone	1	< 1	99% (11%)	21% (21%)	48% (21%)	-15% (9%)	100-500
Methamphetamine	1	< 1	49% (5%)	-10% (8%)	22% (6%)	-13% (4%)	15
Methedrone	1	< 1	68% (3%)	-9% (6%)	33% (34%)	-9% (5%)	-
Methylone	1	< 1	58% (4%)	-17% (9%)	25% (31%)	-17% (4%)	-
Mianserin	1	< 1	84% (6%)	122% (16%)	37% (17%)	36% (9%)	15-70
Midazolam	1	< 1	73% (7%)	-18% (3%)	51% (9%)	-11% (3%)	40-100
Morphine	10	5	56% (5%)	-10% (4%)	19% (41%)	38% (9%)	15 (free form)
Quetiapine	1	< 1	98% (3%)	85% (7%)	57% (13%)	31% (3%)	100-500
Risperidone	1	< 1	107% (11%)	111% (16%)	57% (19%)	30% (10%)	6-20
THC-COOH	10	5	49% (6%)	-53% (6%)	28% (16%)	-41% (6%)	-
Trimipramine	5	1	89% (14%)	166% (26%)	43% (17%)	66% (15%)	10-30
Zolpidem	1	< 1	90% (7%)	32% (4%)	51% (7%)	9% (4%)	80-150

Figures:

Figure 1: 104 real cases have been analyzed by the developed method and two other routine methods. The Venn diagram represents the percentage of identification performed by each method (A). The results were confirmed by quantitative analysis of the substances targeted by all 3 methods. The percentage of identification not performed by the routine approaches is represented depending on the concentration (B).

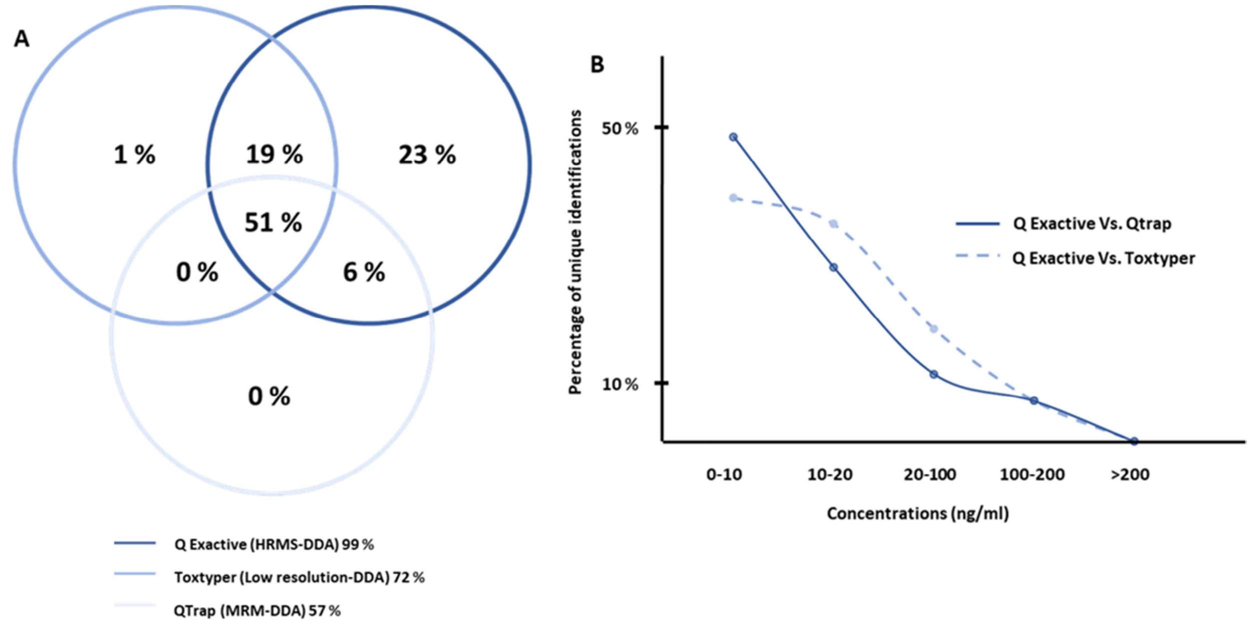
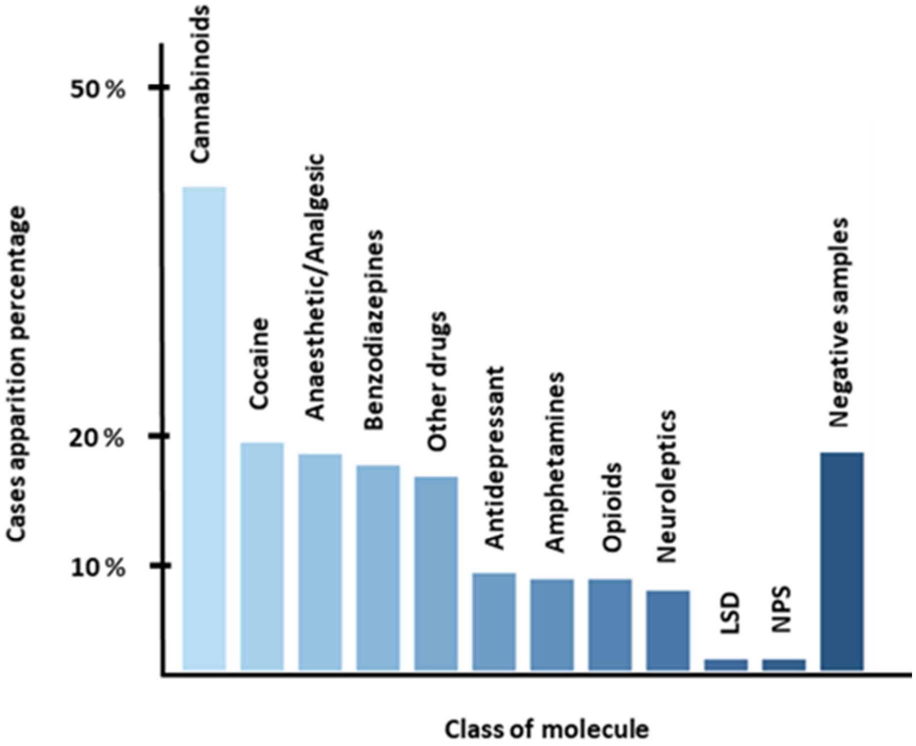


Figure 2: The case apparition percentage in the 104 real cases is represented by bar charts.



Highlights:

- First study on the application of dried blood spot (DBS) microsampling hyphenated with LC and Orbitrap technology for clinical and forensic toxicology screening
- Minimal blood volume requirement for powerful screening anf identification using high resolution MS.
- Validation of the developed method according to guidelines for qualitative approaches and assessment using 104 real cases analysed by reference approaches.
- Great relevance for practice in clinical and forensic toxicology opening new opportunities towards friendly sampling process in non-medical environments.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: