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RESEARCH ARTICLE

INVESTIGATION OF RECENTLY ABUSED DRUGS IN DRIED BLOOD SPOTS USING FTA CARDS BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY: VALIDATION AND APPLICATION TO REAL SAMPLES

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ABSTRACT

A robust and selective liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was established for the analysis of 19 illicit drugs and metabolites in whole blood dried blood spots (DBS) using FTA cards. Samples prepared using Ahlstrom Munksjö Gensaver™ Colorcards were extracted with methanol. Analytical separation of the analytes was maintained using an Agilent Poroshell column with mobile phase A (0.1% formic acid in water) and B (methanol). Multiple Reaction Monitoring (MRM) with positive ionisation on LC-MS/MS was used to develop the method. The method was validated and met the acceptance criteria with acceptable results for carry-over, linearity, specificity, sensitivity, accuracy, precision, matrix effect and recovery. The method was applied to positively reported whole blood samples from patients suspected of drug abuse. Good quantitative agreement was obtained between the DBS and whole blood methods. Application to real DBS samples showed that this method is a good alternative and useful technique for the detection of drugs of abuse in forensic toxicology and appears to provide a good alternative storage condition.

Keywords: *DBS, LC-MS/MS, drug abuse, real samples, illicit drugs*

1. INTRODUCTION

DBS is commonly used as a sampling procedure that involves taking a significantly small volume of blood from the fingertip or heel [1]. DBS is commonly used to collect, store, transport and analyse a variety of human body fluids, and its use has primarily focused on the diagnosis of infections, generally used in the systematic screening of diseases in newborns [2].

DBS provides significant advantages over traditional whole-blood sampling techniques. It is a simple sample collection technique using non-specialist personnel, allowing blood samples to be collected at the point and time of need, a minimally invasive collection procedure, small sample size and integrity of storage and transport. DBS minimizes sample preparation procedures inclusively and simplifies the automation of processes. DBS samples from closed cases can be stored for long periods for clinical and forensic purposes because of the small sample sizes. DBS can also be useful in monitoring

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addiction treatment, preventing the risk of infection with chronic diseases and the risk of viruses such as hepatitis, HIV and blood-borne viruses. The short stability of drugs in biological samples during transport and storage makes interpretation of results difficult. It can also stabilise a wider range of analytes than whole blood samples, even when stored in uncontrolled environmental conditions. [3]. DBS provides a stabilising effect by reducing the hydrolysis reactions of drugs that have ester groups in their chemical structure, such as cocaine and 6-AM [4-5]. Owing to these many benefits, DBS has been accepted for toxicokinetic studies, drug detection and monitoring, analysis of illicit drugs and their metabolites, doping, and assays of molecules with high weight, such as therapeutic proteins, antibodies, biomarkers, and proteomics [1, 5-20].

Since the 1950s, its benefits have been noticed long before [6], preclinical [7] and clinical trials [8, 9] and for drug investigation, drugs or their metabolites abuse [10-31]. Blood is a common biological sample for the detection and quantification of abused drugs and their metabolites in forensic toxicology. DBS has been applied to tetrahydrocannabinol and its main metabolites [18], natural and synthetic cannabinoids [19], cocaine and opiates [21], amphetamines [25] and methadone [28]. However, no validated methods have been published for the detection and quantification of these illicit drugs and/or their metabolites in DBS using gensaver color cards (FTA) with real samples. FTA cards are used to purify and extract nucleic acids for genetic profiling and have never been used in forensic toxicology for the investigation of abused drugs. These cards are only used for genetic profiling and there is no other application to date. The study aims to provide genetic profiling and drug testing in one DBS sample. This is because in some cases it is not possible to take a whole blood sample for drug testing. This study presents a robust, rapid and cost-effective liquid chromatography method for the quantitative detection of 19 illicit drugs and/or their metabolites in DBS using FTA cards. The results were analysed and examined for correlation with those obtained using a validated solid phase extraction whole blood method [32], which was also used to quantify positive results.

2. MATERIAL AND METHODS

2.1. Chemicals and Reagents

ADB-BUTINACA N-butanoic acid, Amphetamine, Methamphetamine, 6-MAM (6-Monoacetylmorphine), ADB-BUTINACA, ADB-BUTINACA N-(4-hydroxybutyl), Benzoylecgonine, Buprenorphine, Cocaine, Codeine, MDMA, MDEA, MDA, MDMB-4en-PINACA, MDMB-4en-PINACA butanoic acid, Methadone, Morphine, THC and THC-COOH were purchased from Chiron (Trondheim, Norway) at concentrations of 1 mg/mL and the internal standard (Diazepam-d5) obtained from Cerilliant (Paloma, TX, USA) at concentrations of 1 mg/mL. All reagents, solutions, and solvents were ammonium acetate, formic acid, methanol, ethyl acetate, and VWR Chemicals (Gibbstown, NJ, USA). Gensaver Color cards were obtained from Ahlstrom Munksjö (Barenstein, Germany).

2.2. LC-MS/MS

The liquid chromatography system included of a Shimadzu liquid chromatography module with a combined column oven (Shimadzu, Kyoto, Japan) maintained at 40 °C and an Agilent Poroshell column (150×4.6 mm, 2.7 µm). The mobile phase included A (5mM ammonium acetate and 0.1 % v/v formic acid in water) and B (methanol). The gradient was set as follows 10% B for 0-0.3 min and then

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B was increased from 10% to 80% within 3 min; between 3-7 min. B was increased from 80% to 95%; B was held at 95% for 7-11 min and decreased from 95% to 10% B for 11.1-15 min. The flow rate, the injection volume and autosampler temperature were set to 0.6 mL/min, 5 µL and 8 °C, respectively. Electrospray ionisation (ESI+) and MRM mode were used for detection. The source voltage was set at 1.5 kV. Nebulizing, heating and drying gas flows were set at 3 L/min, 10 L/min and 10 L/min respectively. The interface, desolvation line, heat block and desolvation temperatures were set at 300 °C, 250 °C, 400 °C and 526 °C respectively. CID gas was set at 270 kPa. The pause time and polarity switching times were set to 1 ms. ms and 5 ms, respectively, and the dwell time for the analytes was set between 5 and 13 ms. The MRM transitions and collision energies were optimized for each transition with infusion. The total run time was 15 minutes.

2.3. Preparation of Standard Solutions

Stock solutions of primary analytes at a concentration of 1 mg/ml were dissolved in methanol and stored at -20°C, and other solutions were prepared daily. The IS was prepared in methanol at a concentration of 200 ng/mL.

2.4. Human Samples

Blank blood samples were used to develop and validate the method and were provided by a regional blood donation centre. Authentic samples were provided from samples collected for routine drug abuse analysis and samples not collected for this study. Thirty samples with 103 positive results for the analytes included in this study and stored at -20°C prior to analysis.

2.5. Sample Preparation

To prepare the samples, 100 µL of sample was applied to Gensaver cards, dried overnight at room temperature and the DBS was then analysed. The whole spot was cut out and placed on a glass slide. Five millilitres of methanol and 50 µL of each deuterated IS working mixture were added. After vortexing for 2 minutes, the tube was gently shaken in a homogeniser for 20 minutes and centrifuged at 4000 rpm for 10 minutes. The extract was evaporated gently at 40 °C under a stream of nitrogen. The residue was reconstituted in 250 µL of a mixture of A (90) and B (10) mobile phases. The sample was vortexed for 2 min, centrifuged at 14,000 rpm for 10 min, transferred to a vial and a 10 µL aliquot was injected into the system.

2.6. Method Validation

The method validation was performed for linearity in terms of selectivity, matrix effect, carryover limit of detection (LOD), limit of quantification (LOQ), linearity, inter and intra-assay precision, recovery, matrix effects and process efficiency. The method has been validated in accordance with international guidelines on forensic toxicology and DBS technique [33, 34]. Six blank blood samples were analysed to investigate the selectivity for potential interferences at the expected retention times of the analytes and IS. Seven calibration points were analyzed between 0.1-50 ng/mL, with three replicates at each concentration. The coefficient of determination (R2) was expected to be greater than 0.995 and the calibrators were expected to quantify within ±10%. The LOD (S/N ≥3) and LOQ (S/N ≥10) were estimated with six fortified samples at the lowest concentrations with acceptable precision and accuracy of <20%. Intra-assay precision was evaluated with six replicates per level concentration at 5 ng/mL (low) and 50 ng/mL (high). Inter-assay precision was assessed over five consecutive days

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using three replicates for each concentration. Carryover was investigated by injecting extracted blanks after five injections of the highest concentration level of the recovery experiment. Recovery, matrix effect and process efficiency were investigated at two different concentrations (n=6, 5 ng/mL (low) and 50 ng/mL (high)) and calculated according to Matuszewski et al. [35].

2.7. Real Sample Analysis

Thirty authentic samples were analysed using the DBS technique and 103 positive results were reported for the analytes included in the study and the results of DBS method were compared with data obtained using the routine whole blood method. In the routine method, the sample volume was 500 µL and the solid phase extraction (SPE) used for sample preparation and analysis was set up by LC-MS/MS analysis [32]. Linear regression analysis, the paired t-test (with a significance level of 0.05) and the Bland-Altman difference plot (with a 95% limit of agreement) were used to investigate the agreement between the results [36-38]. The differences between the two methods and the acceptability of the suitability of the interval were analysed [37].

3. RESULT AND DISCUSSION

3.1. Method Validation Results

The method validation parameters are given in Tables 1 and 2. Selectivity was investigated with six samples. No significant interfering peaks were found. The calibration curve was constructed using the (1/x; x, concentration) linear regression model with a coefficient of determination (R2) of not less than 0.995 and was found to be linear between 0.1-50 ng/mL. The intra- and inter- assay precisions of the method were found to be 1.2-9.9% and 2.1-10.1%, respectively. The accuracy of the method was 91-108%. All precision and accuracy were acceptable. The LODs were 0.3-2.9 ng/mL and the LOQs were 0.4-3.9 ng/mL. No relevant peaks were observed in the blanks used to investigate carryover. Extraction recoveries ranged from 21.1-115.3% at the two concentrations. Matrix effect and process efficiency values were in the range of 83.0-116.0% and 78.0-109.0% at the two concentrations, respectively.

Table 1. Validation data of the developed method (LOD, LOQ, Recovery, Linear Range and Correlation Coefficient values of analytes).

Analyte	LOD (ng/mL)	LOQ (ng/mL)	Recovery (5 ng/mL)	Recovery (50 ng/mL)	Linear Range (ng/mL)	Corr. Coeff. (R2)
6-MAM	0.8	1.1	78.3	77.4	0.1-50	0.999
ADB-BUTINACA	0.3	0.5	102.8	93.8	0.1-50	0.998
ADB-BUTINACA N-(4-hydroxybutyl)	0.3	0.4	98.9	83.7	0.1-50	0.998
ADB-BUTINACA N-butanoic acid	0.3	0.5	97.3	92.2	0.1-50	0.997
Amphetamine	2.6	3.9	25.9	28.3	0.1-50	0.996
Benzoylecgonine	0.7	1.1	115.3	109.2	0.1-50	0.999
Buprenorphine	2.9	3.7	75.8	69.0	0.1-50	0.996

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Cocaine	0.6	0.8	75.0	67.5	0.1-50	0.999
Codeine	0.7	0.8	105.5	101.9	0.1-50	0.996
MDA	1.6	1.9	67.6	62.9	0.1-50	0.996
MDEA	0.6	0.8	63.6	55.8	0.1-50	0.999
MDMA	0.7	1.0	67.1	63.0	0.1-50	0.996
MDMB-4en-PINACA	0.3	0.5	87.0	93.1	0.1-50	0.999
MDMB-4en-PINACA butanoic acid	0.3	0.5	95.9	98.3	0.1-50	0.998
Methadone	0.7	1.2	74.6	64.8	0.1-50	0.997
Methamphetamine	2.0	3.1	24.7	21.1	0.1-50	0.996
Morphine	0.5	0.7	85.3	84.5	0.1-50	0.996
THC	2.3	2.9	63.5	52.0	0.1-50	0.996
THC-COOH	1.0	2.0	91.3	87.3	0.1-50	0.996

Table 1. Validation data of the developed method (recovery and precision values).

Analyte	**Recovery (%) (5 ng/mL)**	**Recovery (%) (50 ng/mL**	**Intra-assay precision (%)(5 ng/mL)**	**Intra-assay precision (%)(50 ng/mL)**	**Inter-assay precision (%) (5 ng/mL)**	**Inter-assay precision (%) 50 ng/mL)**
6-MAM	78.3	77.4	4.8	2.8	5.6	3.1
ADB-BUTINACA	102.8	93.8	3.3	1.9	4.8	2.3
ADB-BUTINACA N-(4-hydroxybutyl)	98.9	83.7	3.8	2.1	4.2	2.5
ADB-BUTINACA N-butanoic acid	97.3	92.2	3.2	2.3	4.1	2.6
Amphetamine	25.9	28.3	5.3	4.5	6.5	5.3
Benzoylecgonine	115.3	109.2	4.3	2.2	5.8	4.3
Buprenorphine	75.8	69.0	5.7	3.0	6.5	5.1
Cocaine	75.0	67.5	4.1	3.0	4.9	3.2
Codeine	105.5	101.9	3.0	2.6	4.1	2.7
MDA	67.6	62.9	4.7	3.7	5.5	2.8
MDEA	63.6	55.8	4.4	2.4	4.9	2.5
MDMA	67.1	63.0	4.3	1.2	6.0	3.4
MDMB-4en-PINACA	87.0	93.1	2.6	1.8	3.8	2.3
MDMB-4en-PINACA butanoic acid	95.9	98.3	2.5	1.7	3.9	2.4
Methadone	74.6	64.8	2.4	1.8	5.6	4.4
Methamphetamine	24.7	21.1	4.1	2.3	4.8	2.1
Morphine	85.3	84.5	3.6	3.3	4.5	3.8
THC	63.5	52.0	9.9	8.8	10.1	9.2
THC COOH	91.3	87.3	3.2	2.3	4.5	2.8

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3.2. Analysis of Real Sample Results

Thirty authentic real collected as part of the Council of Forensic Medicine's drug abuse screening activities, resulting in a total of 103 positive analyte was reported: 6-MAM (n=3), ADB-BUTINACA (n=3), ADB-BUTINACA N-(4-hydroxybutyl) (n=4), ADB-BUTINACA N-butanoic acid (n=4), Amphetamine (n=12), Benzoylecgonine (n=6), Buprenorphine (n=3), Cocaine (n=3), codeine (n=8), MDA (n=5), MDEA (n=3), MDMA (n=5), MDMB-4en-PINACA (n=3), MDMB-4en-PINACA butanoic acid (n=3), methadone (n=4), methamphetamine (n=12), morphine (n=3), THC (n=3), THC-COOH (n=11). The results of DBS and whole blood analyses are shown in Table 3.

Table 2. Concentrations of analytes on DBS and classical whole blood method.

Compound	DBS Concentration (ng/mL)	Case	Classical Whole Blood Method (ng/mL)	Case
6-MAM	0.9-2.7	3	1.3-3.2	3
ADB-BUTINACA	0.9-1.2	3	1.1-1.4	3
ADB-BUTINACA N-(4-hydroxybutyl)	2.3-3.9	4	2.8-4.1	4
ADB-BUTINACA N-butanoic acid	8.3-11.2	4	9.1-12.3	4
Amphetamine	9.5-68.9	12	3.0-75.8	12
Benzoylecgonine	1.4-101.6	6	2.0-121.3	6
Buprenorphine	6.3-7.8	3	7.1-9.3	3
Cocaine	3.2-4.6	3	4.3-5.9	3
Codeine	0.9-5.1	8	0.8-4.3	8
MDA	6.4-15.5	5	7.4-14.6	5
MDEA	2.7-4.3	3	2.3-4.1	3
MDMA	70.7-107.1	5	65.9-103.4	5
MDMB-4en-PINACA	0.6-1.8	3	0.7-1.7	3
MDMB-4en-PINACA butanoic acid	2.8-10.3	3	3.1-9.7	3
Methadone	6.9-14.8	4	8.3-14.8	4
Methamphetamine	4.6-195.2	12	3.2-183.6	12
Morphine	0.7-10.7	8	1.7-8.9	8
THC	3.1-4.9	3	5.6-7.3	3
THC COOH	4.5-43.1	11	6.6-39.2	11

All positive analytes were detected by routine methods and DBS. DBS was found to be completely reliable when compared with the routine whole blood method and no false negatives or false positives were observed. The total ion chromatograms and product ion spectra of DBS and whole blood from the same sample are shown in Figure 1.

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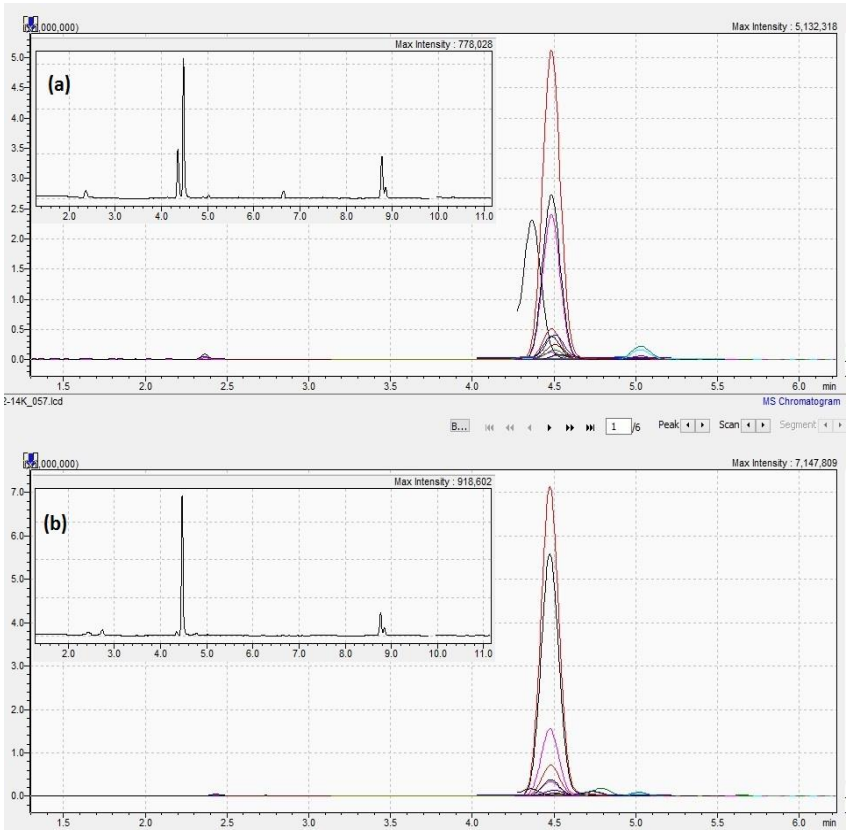

Figure 1. The total ion chromatogram and extracted ion chromatograms of whole blood method (a) and DBS method (b) of the same case sample.

The analyte concentrations obtained by the DBS method were found to be compatible with the routinely analysed whole blood method and a strong correlation was found between the results (Pearson's r = 0.9650). In addition, a significant correlation was found using least squares regression analysis ($p < 0.05$), with r2 = 0.9406. A paired two-tailed t-test with a significance level of 0.05 was also used to test for differences between the two methods and no significant difference was found ($t_{calculated} < t_{table}$). Finally, the Bland-Altman analysis was also performed between the two groups (limits of agreement of 95%; lower and upper limits, median ±1.96×sd (standard deviation)). The plot shows a good agreement between the two methods. The differences between the THC-COOH values were examined between two methods using the Bland-Altman difference plot (Figure 2).

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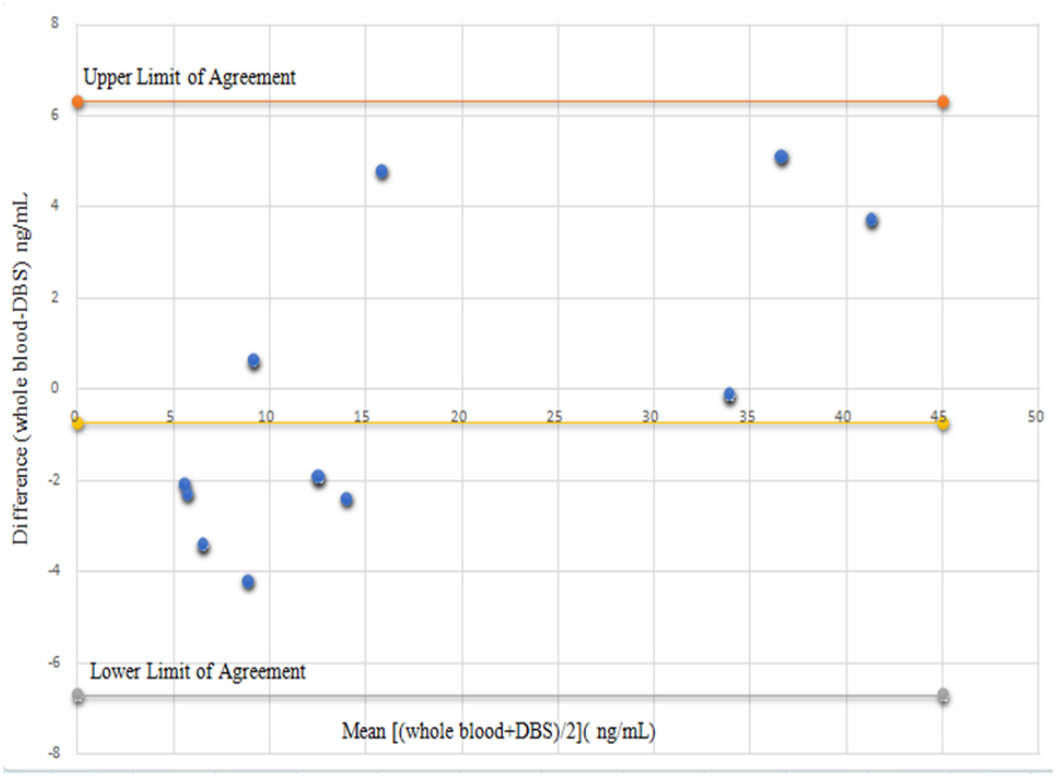

Figure 2. Bland-Altman difference plots of the differences between DBS and whole blood assays against the average obtained by the two assays (Upper Limit Agreement=median+1.96×sd and Lower Limit Agreement=median-*1.96*×sd).

This study confirms and extends the findings of previously published methods [2, 3, 4, 18, 19, 20, 23, 25, 27, 28, and 29]. In particular, an evaluation of the stability of analytes between the two methods showed that the DBS method produced more reliable quantitative results than the classical methods, even though the cards were stored at room temperature after sampling [19, 20, 23, 25, 27, 28, and 29].

DBS samples provide a compatible alternative for the detection of abused drugs. The method is fast, reliable and selective. It can be a good alternative to complex sample preparation methods such as solid phase extraction (SPE). Material and solvent consumption can be reduced, and smaller sample volumes (100 µL or less) can be easily handled. This study proved that analyses by DBS technique are as sensitive and reliable as classical methods and can be a good alternative method.

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4. CONCLUSION

The LC-MS/MS method developed and validated successfully for the determination and quantification of 19 abused drugs in DBS using FTA cards. Method validation results showed compliance with the guidelines. This study is the first to investigate classical abused drugs and emerging synthetic cannabinoids in authentic whole blood samples of drug abuse suspects collected on DBS. This study confirmed that DBS cards are robust and safe sample storage for the analysis of most drugs analyzed in whole blood. The results obtained from 30 authentic cases showed a promising qualitative and quantitative compliance between the whole blood and the same blood samples dried on DBS cards. In this study, the developed method represents a good complementary alternative to forensic sample analysis by providing a uncomplicated, inexpensive and easy-to-maintain method for determination and quantification simultaneously of abused drugs in the field of forensic toxicology. According to the literature, the stability of most drugs in DBS, even without the addition of preservatives, allows for robust and accurate quantitative results after sample collection and storage at room temperature. With these promising technical, sampling, storage and stability advantages, new research is needed in relation to different applications such as occupational and clinical toxicology and therapeutic monitoring.

CONFLICT OF INTEREST

The author declares no conflict of interests.

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REFERENCES

[1] Sadler Simões, S., Castañera Ajenjo, A., and Dias, M.J. (2018). Dried blood spots combined to an UPLC-MS/MS method for the simultaneous determination of drugs of abuse in forensic toxicology. J Pharm Biomed Anal, 5,147,634-644.

[2] Grüner, N., Stambouli, O., and Ross, R.S. (2015). Dried blood spots preparing and processing for use in immunoassays and in molecular techniques. J Vis Exp, 13(97), 52619.

[3] Quraishi, R., Jain, R., and Ambekar, A. (2013). The use of dried blood spot samples in screening drugs of abuse. Pharmacology & Pharmacy, 4(2), 152-159.

[4] Alfazil, A.A., and Anderson, R.A. (2008). Stability of benzodiazepines and cocaine in blood spots stored on filter paper. J Anal Toxico, 32(7), 511-515.

[5] Garcia Boy, R., Henseler, R., Mattern, R., and Skopp, G. (2008). Determination of morphine and 6-acetylmorphine in blood with use of dried blood spots. Ther Drug Monit, 30(6), 733-739.

[6] Beaudette, P., and Bateman, K.P. (2004). Discovery stage pharmacokinetics using dried blood spots, J Chromatogr B Analyt Technol Biomed Life Sci. 809(1), 153-158.

[7] Barfield, M., Spooner, N., Lad, R., Parry, S., and Fowles, S. (2008). Application of dried blood spots combined with HPLC-MS/MS for the quantification of acetaminophen in toxicokinetic studies. J Chromatogr B Analyt Technol Biomed Life Sci, 870(1), 32-37.

[8] Spooner, N., Lad, R., and Barfield, M. (2009). Dried blood spots as a sample collection technique for the determination of pharmacokinetics in clinical studies: considerations for the validation of a quantitative bioanalytical method. Anal Chem, 81(4), 1557-1563.

[9] Dainty, T.C., Richmond, E.S., Davies, I., and Blackwell, M.P. (2012). Dried blood spot bioanalysis: an evaluation of techniques and opportunities for reduction and refinement in mouse and juvenile rat toxicokinetic studies. Int J Toxicol, 31, 4.

[10] Velghe, S., Troyer, R.D., and Stove, C. (2018). Dried blood spots in therapeutic drug monitoring and toxicology. Expert Opin Drug Metab Toxicol, 14, 1-3.

[11] Edelbroek, P.M., Van der Heijden, J., and Stolk, L.M. (2009). Dried blood spot methods in therapeutic drug monitoring: methods, assays, and pitfalls. Ther Drug Monit, 31, 327.

[12] McDade, T.W., Williams, S., and Snodgrass, J.J. (2007). What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. Demography, 44, 899.

[13] Den Burger, J.C., Wilhelm, A.J., Chahbouni, A., Vos, R.M., Sinjewel, A., and Swart, E.L. (2012). Analysis of cyclosporin A, tacrolimus, sirolimus, and everolimus in dried blood spot samples using liquid chromatography tandem mass spectrometry. Anal Bioanal Chem, 404, 1803.

[14] Versace, F., Déglon, J., Lauer, E., Mangin, P., and Staub, C. (2013). Automated DBS extraction prior to HILIC/RP LC–MS/MS target screening of drugs. Chromatographia, 76(19-20), 1281–1293.

[15] Thomas, A., Deglon, J., Steimer, T., Mangin, P., Daali, Y., and Staub, C. (2010). On-line desorption of dried blood spots coupled to hydrophilic interaction/reversed-phase LC/MS/MS system for the simultaneous analysis of drugs and their polar metabolites. J Sep Sci, 33(6-7), 873-879.

[16] Moretti, M., Freni, F., Tomaciello, I., Vignali, C., Groppi, A., Visonà, S.D., Tajana, L., Osculati, A.M.M., and Morini, L. (2019). Determination of benzodiazepines in blood and in dried blood spots collected from post-mortem samples and evaluation of the stability over a three-month period. Drug Test Anal, 11, 1403–1411.

[17] Moretti, M., Freni, F., Tomaciello, I., Vignali, C., Groppi, A., Visonà, S.D., Osculati, A.M.M., and Morini, L. (2019). Determination of antidepressants and antipsychotics in dried blood spots (DBSs) collected from post-Mortem samples and evaluation of the stability over a three-month period. Molecules, 24, 3636.

[18] Mercolini, L., Mandrioli, R., Sorella, V., Somaini, L., Giocondi, D., Serpelloni, G., and Raggi, M.A. (2013). Dried blood spots: liquid chromatography-mass spectrometry analysis of Δ9-tetrahydrocannabinol and its main metabolites. J Chromatogr A, 4, 1271(1), 33-40.

[19] Protti, M., Rudge, J., Sberna, A.E., Gerra, G., and Mercolini, L. (2017). Dried haematic microsamples and LC-MS/MS for the analysis of natural and synthetic cannabinoids. J Chromatogr B Analyt Technol Biomed Life Sci, 1044-1045, 77-86.

[20] Ambach, L., Redondo, A.H., König, S., and Weinmann, W. (2013). Rapid and simple LC-MS/MS screening of 64 novel psychoactive substances using dried blood spots. Drug Test Anal, 6(4), 367-375.

[21] Antelo-Domínguez, A., Cocho, J.A., Tabernero, M.J., Bermejo, A.M., Bermejo-Barrera, P., and Moreda-Piñeiro, A. (2013). Simultaneous determination of cocaine and opiates in dried blood spots by electrospray ionization tandem mass spectrometry. Talanta, 117, 235-241.

[22] Jantos, R., Veldstra, J.L., Mattern, R., Brookhuis, K.A., and Skopp, G. (2011). Analysis of 3,4-methylenedioxymethamphetamine: whole blood versus dried blood spots. J Anal Toxicol, 35(5), 269-273.

[23] Clavijo, C.F., Hoffman, K.L., Thomas, J.J., Carvalho, B., Chu, L.F., Driver, D.R., Hammer, G.B., Christians, U., and Galinkin, J.L. (2011). A sensitive assay for the quantification of morphine and its active metabolites in human plasma and dried blood spots using high performance liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem, 400(3), 715-728.

[24] Mommers, J., Mengerink, Y., Ritzen, E., Weusten, J., Van der Heijden, J., and Van der Wal, S. (2013). Quantitative analysis of morphine in dried blood spots by using morphine-d3 pre-impregnated dried blood spot cards. Anal Chim Acta, 774, 26-32.

[25] Saussereau, E., Lacroix, C., Gaulier, J.M., and Goulle, J.P. (2012). On-line liquid chromatography/tandem mass spectrometry simultaneous determination of opiates, cocainics and amphetamines in dried blood spots. J Chromatogr B Analyt Technol Bimed Life Sci, 885(886), 1-7.

[26] Sosnoff, C.S., Ann, Q., Bernert, J.T., Powell, M.K., Miller, B.B., Henderson, L.O., Hannon, W.H., Fernhoff, P., and Sampson, E.J. (1996). Analysis of benzoylecgonine in dried blood spots

by liquid chromatography– atmospheric pressure chemical ionization tandem mass spectrometry. J Anal Toxicol, 20(3), 179–184.

[27] Thomas, A., Geyer, H., Schänzer, W., Crone, C., Kellmann, M., Moehring, T., and Thevis, M. (2012). Sensitive determination of prohibited drugs in dried blood spots (DBS) for doping controls by means of a benchtop quadrupole/Orbitrap mass spectrometer. Anal Bioanal Chem, 403(5), 1279-1289.

[28] Saracino, M.A., Marcheselli, C., Somaini, L., Pieri, M.C., Gerra, G., Ferranti, A., and Raggi, M.A. (2012). A novel test using dried blood spots for the chromatographic assay of methadone. Anal Bioanal Chem, 404(2), 503-511.

[29] Moretti, M., Visonà, S.D., Freni, F., Tomaciello, I., Vignali, C., Groppi, A., Tajana, L., Osculati, A.M.M., and Morini, L. (2018). A liquid chromatography-tandem mass spectrometry method for the determination of cocaine and metabolites in blood and in dried blood spots collected from postmortem samples and evaluation of the stability over a 3-month period. Drug Test Anal, 10, 1430–1437.

[30] Moretti, M., Manfredi, A., Freni, F., Previderé, C., Osculati, A.M.M., Grignani, P., Tronconi, L., Carelli, C., Vignali, C., and Morini, L. (2021). A comparison between two different dried blood substrates in determination of psychoactive substances in postmortem samples. Forensic Toxicol, 39, 385–393.

[31] Kacargil, C.U., Daglioglu, N., and Goren, I.E. (2020). Determination of illicit drugs in dried blood spots by lc–ms/ms method: validation and application to real samples. Chromatographia, 83(7), 885-892.

[32] Yeter, O., and Erol Öztürk, Y. (2019). Detection and quantification of 5F-ADB and its methyl ester hydrolysis metabolite in fatal intoxication cases by liquid chromatography-high resolution mass spectrometry. Forensic Sci Int, 302, 109866.

[33] Scientific Working Group for Forensic Toxicology. (2013). Standard practices for method validation in forensic toxicology. J Anal Toxicol, 37(7), 452-74.

[34] Timmerman, P., White, S., Globig, S., Lüdtke, S., Bruner, L., and Smeraglia, J. (2011). EBF recommendation on the validation of bioanalytical methods for dried blood spots. Bioanalysis, 3(14), 1567-1575.

[35] Matuszewski, B.K., and Constanzer, M.L. (2003). Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC – MS/MS. Anal Chem, 75, 3019–3030.

[36] Bland, J.M., and Altman, D.G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. Lancet, 1(8476), 307-310.

[37] Stockl, D., Dewitte, K., and Thienpont, L. (1998). Validity of linear regression in method comparison studies: is it limited by the statistical model or the quality of the analytical input data? Clin Chem, 44(11), 2340–2346.

[38] Dewitte, K., Fierens, C., Stockl, D., and Thienpont, L. (2002). Application of the Bland-Altman plot for interpretation of method- comparison studies: a critical investigation of its practice. Clin Chem, 48(5), 799–801.