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On-line liquid chromatography/tandem mass spectrometry simultaneous determination of opiates, cocainics and amphetamines in dried blood spots

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ABSTRACT

A novel approach has been developed for the illicit drugs quantitative determination using dried blood spots (DBS) on filter paper. The illicit drugs tested were opiates (morphine and its 3- and 6-glucuronide metabolites, codeine, 6-monoacetylmorphine), cocainics (ecgonine methylester, benzoylecgonine, cocaine, cocaethylene) and amphetamines (amphetamine, methamphetamine, MDA, MDMA, MDEA). The described method, requiring a small blood volume, is based on high performance liquid chromatography coupled to tandem mass spectrometry using on-line extraction. A Whatman card 903 was spotted with 30 μL of whole blood and left overnight to dry at room temperature. A 3-mm diameter disk was removed using a manual punch, suspended in 150 μL of water for 10 min with ultrasonication, and then 100 μL was injected in the on-line LC–MS/MS system. An Oasis HLB was used as an extraction column and a C18 Atlantis as an analytical column. The chromatographic cycle was performed with 20 mM ammonium formate buffer (pH 2.8) (solvent A) and acetonitrile/solvent A (90:10, v/v) gradient in 16 min. Detection was performed in positive electrospray ionization mode (ESI+) with a Quattro Micro (Waters). Recoveries of all analytes were up to 80%. DBS were stored in duplicate at 4 °C and −20 °C for up to 6 months. Illicit drugs seemed to be much more stabled at −20 °C. Furthermore, it was tested whether analysis of DBS may be as reliable as that of whole blood investigating authentic samples; significant correlations were obtained. This DBS assay has potential as rapid, sensitive and inexpensive option for the illicit drugs determination in small blood volumes, which seems of great interest in suspected cases of driving under the influence of drugs.

1. Introduction

Determination of illicit drugs in biological fluids is of great importance in many fields. The list of drugs of abuse can vary, depending on the context in which the samples are analyzed. Clinical and forensic toxicology, workplace drug testing, testing of driving under the influence of drugs, doping analysis and rehabilitation programs, all focus on different drugs of abuse: opiates, cocainics, amphetamines. As more potent drugs of abuse are being produced, it is evident that lower levels of sensitivity will be required, thus new analytical strategies will have to take this trend into account. We believe that it would be useful to develop a LC–MS/MS method that could detect a wide ranging illicit drugs simultaneously using relatively simple sample clean-up procedure as on-line solid phase extraction [1]. An on-line LC–MS/MS method was developed and validated for opiates, cocainics and amphetamines quantitative determination in serum and plasma [2]. The first described method was adapted to be more efficient for complex matrices as whole blood. The main evolution concerned the sample preparation. The whole blood deproteinization step was replaced by a simple dilution in water to 1:50. We believe that it would be of great interest to apply this sample pre-analytical treatment to dried blood spots (DBS). The use of DBS has successfully been introduced in neonatal metabolic screening. Over the past decade, many applications have been reported for both qualitative and quantitative screening of metabolic disorders [3–5]. Therapeutic drug monitoring with dried blood spot sampling has been reported for several drugs [6–11], as pharmaco-toxicokinetic studies [12,13] and illicit drugs determination [14–17]. DBS offers a number of advantages, now well established, over conventional whole blood sample collection [13]. First, it requires a less invasive sampling method, DBS being obtained via a finger prick. Second, it offers a simple storage and easier transfer because there is no requirement for freezers or dry ice in most applications. Third, DBS requires a smaller blood volume, less than 100 μL compared with more than 0.5 mL blood which is usually obtained for conventional blood sample collection. DBS seems to be an alternative to conventional blood sample, the actual trends

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in biomedical analysis being to reduce biological materials, time and analysis costs. In the main described methods, the DBS extraction is conventional with an organic solvent, containing an internal standard. This work presents a LC–MS/MS method allowing simultaneous opiates, cocainics and amphetamines determination in whole blood using dried blood spots, with an extraction carried out on-line.

2. Experimental

2.1. Chemicals and reagents

Methanol and acetonitrile, both HPLC gradient grade, were purchased from Carlo Erba (Italy). All other chemicals, formic acid (99%, Carlo Erba Reagenti, Italy), ammonia solution (25%, Merck), ammonium formate (Fluka, Buchs, Switzerland) were of analytical grade. Deionized water was purified using the Milli-Q-system from Millipore Corporation (Bedford, MA, USA). Drug free whole blood was purchased from Utak (Valencia, California, USA).

Individual stock solutions of the drugs and their deuterated analogues:

Opioids: morphine, β3- and β6-morphine-glucuronide (M3G and M6G), codeine and 6-monoacetylmorphine (6-MAM) were purchased from Promochem (Molsheim, France).

Cocainics [cocaine, ecgonine methylester (ME), benzoylecgonine (BE), cocaethylene], amphetamines (amphetamine, methamphetamine, MDA, MDMA, MDEA) and deuterated analogues were purchased from Promochem.

A working solution of non-deuterated compounds at 10 μg/mL in methanol and stored at −20 °C, was used for standards preparation. For 6-acetyl-morphine, working solution was prepared in acetonitrile. Standard solutions were prepared from working solution diluted with drug-free whole blood, of 5–10–50–100–200 ng/mL. A mixed internal deuterium labelled standards working solution of 100 ng/mL was prepared in water.

2.2. LC–MS/MS system

The column-switching LC–MS/MS system consisted of a 1525 Micro Binary Pump (pump 1 for wash solvent; Waters, Milford, MA, USA), an Alliance 2795 HPLC pump (pump 2 for elution mobile phase; Waters), a sample injection valve with 100 μL sample loop (Rheodyne, Cotati, CA, USA), a 10-port valve allowing switching or direct injection (valve V1; Rheodyne), a six-port switching valve (valve V2; Rheodyne), and a Quattro Micro™ tandem mass spectrometer (Waters) used in positive electrospray ionization (ESI) mode and controlled by computer using MassLynx software (Version 4.1). The extraction column was an Oasis HLB (2.1 × 20 mm; 25 μm, Waters). The reversed-phase analytical column was an Atlantis C18 (2.1 × 150 mm; 3 μm, Waters). The wash solvent for sample extraction and enrichment, delivered by pump 1, was Milli-Q water fortified with 0.2% ammonia. The mobile phase for sample elution and separation, delivered by pump 2, consisted of a solvent A (20 mM ammonium formate aqueous solution adjusted to pH 2.8 with formic acid) and a solvent B (acetonitrile–solvent A, 90–10; v/v). The mass spectrometer conditions for the quantitative analysis were the same as determined above [2]: probe capillary voltage was 3.2 kV; the source block and desolvation temperatures were 120 °C and 450 °C, respectively. Two mass transitions were selected for each analyte, the most intense being used for quantification and the other for confirmation. Cone voltages, collision energies, as precursor and product ion transitions, are also the same as those described in the reference method [2]. Quantitative measurements were carried out in the multiple reaction monitoring mode.

2.3. Column-switching procedure

The column switching integrated procedure steps were: sample injection, extraction and transfer of the analytes fraction followed by chromatographic separation. Valve 1 and pump 1 were used to load 100 μL of diluted sample fortified with deuterium labelled internal standards, onto the Oasis column. The wash solvent of pump 1 was delivered to the extraction column at a flow rate to 2 mL/min. Analytes were retained on the Oasis column, while matrix compounds were flushed out to waste with the wash solvent. After 1 min, the matrix had been fully washed out of the extraction column; valve 2 was switched into transfer position, thereby coupling the extraction and the analytical columns. Analytes were rapidly eluted from the extraction column by back-flushing at a flow rate of 0.3 mL/min. The gradient applied for the chromatographic analysis was as follows: 0.00–6.00 min: gradient increase to 50% B, 6.01–6.50 min: gradient increase to 95% B, 6.51–10.00 min: 95% B, 10.01–10.50 min: gradient decrease to 8% B and 10.51–16 min: 8% B for re-equilibration of the HPLC column (Fig. 1). In parallel, the 6-port valve was switched back to initial position and the Oasis was washed out with methanol/water/formic acid (90/9.8/0.2; v/v) for 2 min with a flow rate of 1 mL/min and subsequently re-equilibrated with aqueous ammonia solution. The analytical column was maintained at 45 °C in a column oven. Total analysis cycle time including SPE and separation was established at 16 min.

3. Sample preparation

3.1. Whole blood sample

100 μL of whole blood was diluted to 1:10 with 100 μL of working deuterated standards solution and 800 μL of water. After vortex-mixed and 5 min centrifugation (13,500 × *g*) to remove an eventual precipitation, 400 μL of water was added to 100 μL of the supernatant. The final concentration of deuterium labelled internal standards was 1 ng/mL. 450 μL was transferred into the HPLC vials and placed in the auto sampler. 100 μL is injected in the LC–MS/MS system.

3.2. Dried blood spots

30 μL of whole blood sample was spotted on a filter paper Whatman 903® (Whatman, Dassel, Germany). The blood spots were allowed to dry overnight at room temperature, protected from light and humidity. A 3-mm diameter disk was removed from the center of sample area using a manual punch and was suspended in 150 μL of water containing 1 ng/mL of deuterated internal standards. After 10 min ultrasonication, 100 μL was injected in the on-line LC–MS/MS system.

4. Assay validation

4.1. Whole blood

Linearity, interassay precision and accuracy were assessed analyzing a set of calibrants at five concentration values ranging from 5 to 200 ng/mL each day for 5 days. Calibrants included the same 14 compounds as those validated in serum and plasma [2]: morphine and its glucuronide metabolites, codeine, 6-MAM, cocaine, cocaethylene, benzoylecgonine, ecgonine methylester, amphetamine, methamphetamine, MDA, MDMA and MDEA. The regression line was calculated using a weighted [1/(concentration)^2] least-squares regression model. The intraassay precision and accuracy were determined at 2 concentration

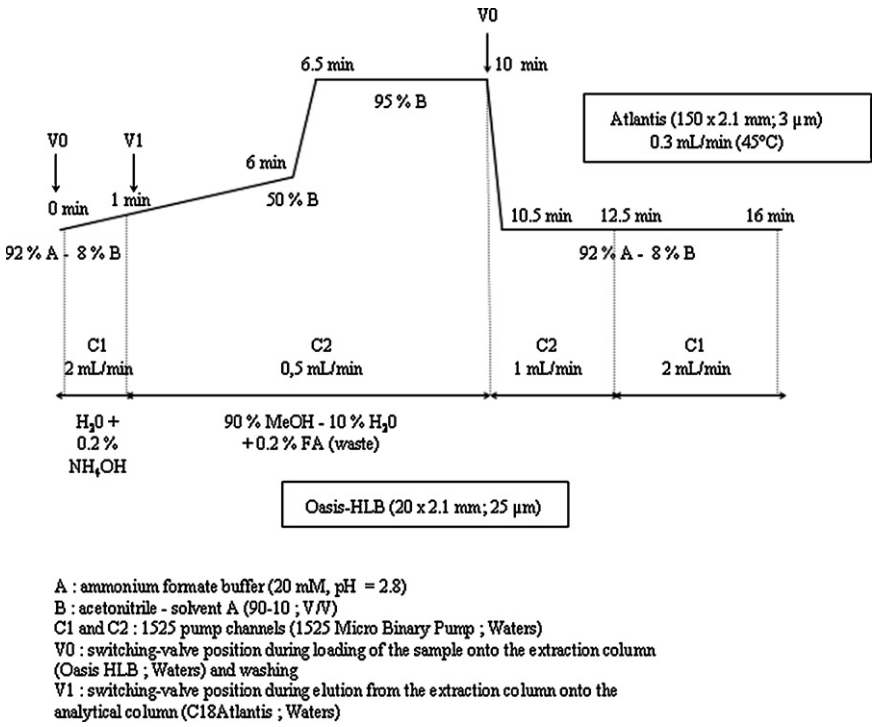

Fig. 1. *At the top*: analytical gradient (C18 Atlantis column). *At the bottom*: sample extraction and enrichment, and analytes back-flushing from the Oasis column.

levels (10 and 50 ng/mL), with 5 spiked whole blood samples for each level analyzed on the same day. The sensitivity of the method was evaluated with the limit of detection (LOD) and the limit of quantification (LOQ). LOD was defined as the concentration with a signal-to-noise ratio of at least 3, while LOQ was the lowest standard with a signal-to-noise ratio of at least 10 and acceptable precision and accuracy. Acceptable limits for precision and accuracy were set at a relative standard deviation (RSD) and percentage difference from the expected value (bias) of less than 15%. Extraction recovery were assessed comparing the peak-area ratios of the compounds spiked at the same concentration in whole blood and in water after column-switching injection, which was in fact the assessment of the matrix effects rather than that of extraction recovery [18]. Ion suppression was investigated injecting 3 different blank whole blood samples, while in parallel, a mixture of the compounds at 5 μg/mL prepared in 20 mM ammonium formate–solvent B (1 v/3 v) was continuously infused at a flow rate of 20 μL/min in the ionization source through a Tee Piece.

4.2. Dried blood spots

The main application of the illicit drugs quantitative analysis in DBS being suspected driver under the influence of drugs controls, only 9 compounds were evaluated: morphine, codeine, cocaine, benzoylecgonine, ecgonine methylester, amphetamine, methamphetamine, MDA and MDMA. The whole blood levels of these substances are frequently the only data taken into account by the Justice Authorities in France. In order to investigate if interfering endogenous compounds are present, blank blood spots, spiked blood spots and blood spots obtained from whole blood samples done in suspected driver under the influence of drugs (DUID) controls and positive for illicit drugs. These last whole blood samples were analyzed initially in LC–MS/MS for Justice's authority, and secondary for the assay validation, after spotting onto paper card. The recovery of analytes was determined by comparing the peak area of desorpted DBS to this of undesorpted samples. Five replicate spiked samples at 20 ng/mL were examined. Samples are defined as: A, samples with no matrix and no desorption (compounds spiked in water); B, samples with matrix but without extraction (compounds spiked after DBS desorption in water); and C, samples with matrix and extraction (compounds spiked in whole blood before entire procedure). Then, $C/B \times 100$ gives the extraction recovery while $C/A \times 100$ represents overall method recovery. Moreover, the matrix effect will be given by $(B/A - 1) \times 100$. The stability of analytes in DBS was evaluated with spiked samples at 10 and 50 ng/mL at 4 °C and −20 °C, up to 6 months ($n = 5$ for each analyte, time point, temperature and concentration). As for whole blood samples, matrix effects were evaluated with ion suppression assay.

4.3. Comparison of whole blood and dried blood spots using authentic samples

The comparison between whole blood and dried blood spots was made with the collaboration of an other forensic toxicology laboratory (CHU Dupuytren, Limoges, France). Twenty authentic whole blood samples, corresponding to blood samples realized initially from drivers suspected of driving under the influence of drugs, under Justice's authority for forensic expertise. Secondary, these samples were used in our study after the expertise phase, by spiking Whatman paper card with 30 μL of whole blood. After the time required to dry DBS, whole blood samples and dried blood spots were send to our laboratory by mailing, fresh whole blood samples being transported at 4 °C. Fresh whole blood and DBS samples were simultaneously analyzed in on-line LC–MS/MS.

5. Results

5.1. Whole blood samples: LC–MS/MS assay validation

All analytes were separated with the chromatographic run-time of 16 min (Fig. 2). This complete chromatographic separation of all illicit drugs and deuterated analogues permitted using 3 MRM timed windows and optimizing the dwell times as well as the sensitivity. Each MRM function contained no more than 19 $[M+H]^+$

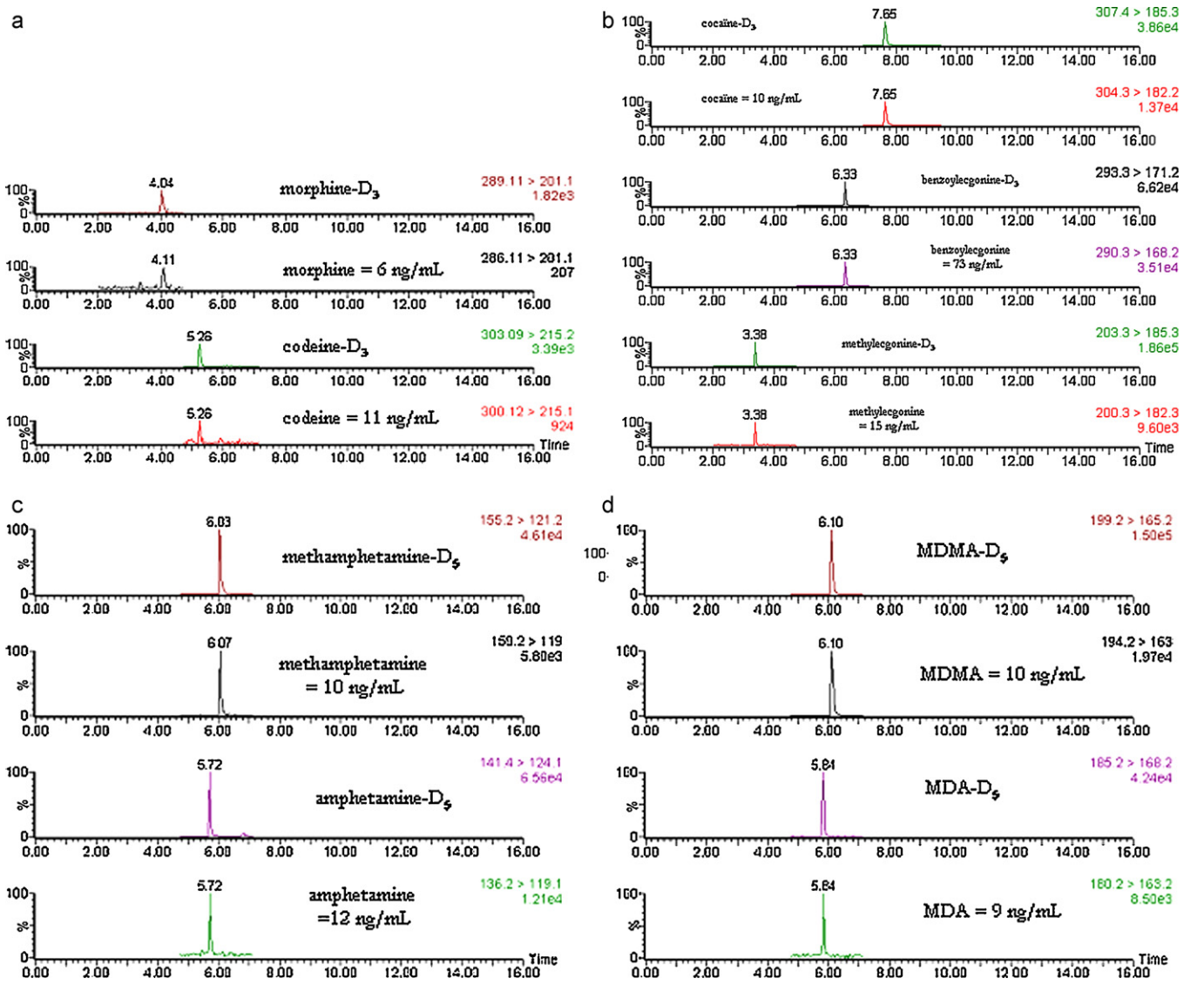

Fig. 2. Dried blood spots: representative ion chromatograms of opiates (a), cocainics (b) and amphetamines (c and d).

transitions. The quantification is obtained for all molecules and deuterated analogues in an only chromatographic run. Retention times were the same as described in serum for opiates and ecgonine methylester [2]. For amphetamines and other cocainics, retention times increased about 0.4 min. The relationships between analytes concentrations and peak area ratios were linear over the range 5–200 ng/mL. For glucuronides, the method was linear from 10 to 200 ng/mL. The linear correlation coefficients (r^2) values were better than 0.99 for all analytes. The limits of detection (LOD), based on the calculation of signal-to-noise ratios equal to 3, ranged between 0.5 and 2 ng/mL, and were up to 5 ng/mL for glucuronides (Tables 1 and 2). The limits of quantification (LOQ) corresponded to the first calibration point at 5 ng/mL. As shown in Tables 1 and 2, the method presented satisfying precision and accuracy. The relative

Table 1
Opiates assay validation parameters in whole blood.

Compound	LOD (ng/mL)	Precision[a] (RSD %, $n = 5$)		Accuracy[a] (bias %, $n = 5$)	
		Intra-assay	Inter-assay	Intra-assay	Inter-assay
Morphine	2.0	6.3	9.9	7.4	5.2
		3.4	5.3	5.0	1.6
6-MAM	1.0	6.4	5.4	1.9	1.0
		5.5	2.5	−2.4	−4.1
Codeine	2.0	8.6	3.1	0.8	5.8
		3.7	2.2	−0.8	−1.2
M3G	5.0	13.0	9.8	5.7	3.1
		7.8	3.7	−2.0	−0.4
M6G	5.0	12.7	11.0	13.0	11.6
		8.0	8.5	5.4	−1.3

[a] Precision and accuracy were assessed at 10 ng/mL and 50 ng/mL.

Table 2
Cocainics and amphetamines assay validation parameters in whole blood.

Compound	LOD (ng/mL)	Precision[a] (RSD %, $n=5$)		Accuracy[a] (bias %, $n=5$)	
		Intra-assay	Inter-assay	Intra-assay	Inter-assay
Cocaine	1.0	3.6	3.0	2.9	−0.8
		2.9	2.2	−2.4	−1.5
Coca-ethylene	1.0	3.6	3.5	−1.8	0.8
		1.9	3.3	−0.5	−1.1
Ecgonine methylester	0.5	4.2	8.0	6.4	4.6
		5.3	3.3	1.4	−0.6
Benzoyl-ecgonine	0.5	2.7	2.9	−0.6	−0.6
		2.7	3.2	2.4	0.8
Amphe-tamine	0.5	0.8	2.6	4.2	1.4
		2.3	1.2	2.2	−0.5
Metamphe-tamine	0.5	4.2	2.1	4.4	2.3
		2.5	3.0	2.2	−0.6
MDA	1.0	5.6	5.2	0.6	−1.0
		5.5	2.2	−0.1	1.3
MDMA	1.0	5.2	4.5	−1.4	−2.9
		1.1	3.9	0.5	3.4
MDEA	0.5	7.0	4.4	−1.0	0.6
		3.0	2.9	0.8	−0.7

[a] Precision and accuracy were assessed at 10 ng/mL and 50 ng/mL.

Table 3
Extraction and overall DBS method recoveries.

Compound	Recoveries[a] (%, $n=5$)	
	Extraction	Method
Morphine	90	90.2
Codeine	93.2	107.3
Cocaine	100	81.6
Ecgonine methylester	85.9	81.4
Benzoyl-ecgonine	99.4	78.9
Amphe-tamine	97.4	96.7
Metamphe-tamine	88.6	90.3
MDA	121.6	129.5
MDMA	101.6	98.1

[a] Recoveries were assessed at 20 ng/mL.

standard deviations (RSD %) for intra- and interday were respectively in the range 0.8–8.6 (up to 13% for glucuronides) and 1.2–9.9 (up to 11% for glucuronides). The bias (%) for intra-and interday ranged from −2.4 to 7.4 (up to 13% for glucuronides) and −4.1 to 5.8% (up to 11.6% for glucuronides). The recoveries of all analytes were respectively up to 80% for opiates and amphetamines and 90% for cocainics. As described above for serum and plasma [2], no ion suppression effect was observed at the retention time of the analytes.

5.2. Dried blood spots: specificity, method recoveries, matrix effects and analytes stability

Under the assay conditions described above, no interfering peaks were observed in the blank chromatograms (10 blanks were examined), demonstrating the method specificity. The extraction and overall method recoveries were determined at 20 ng/mL ($n=5$) using equations described above. The extraction recoveries for all analytes were in the range 85.9–121.6% and, as shown in Table 3, the overall method recoveries ranged from 78.9 to 129.5%. Calculated matrix effects varied from −3.5 to 1.9, demonstrating no significant enhancement or suppression from matrix. Similarly, investigation according to Matuszewski et al. [19] demonstrated no ion suppression or enhancement at the analytes retention times. The stability of the compounds was determined at two storage temperatures, 4 °C and −20 °C, by measuring the drug content remaining in DBS (in duplicate) after storing for 48 h, 1 month, 3 months and up to 6 months, and comparing with the initial concentrations, 10 and 50 ng/mL. The percentages of initial concentrations after 6 months storage are presented in Table 4. For all analytes, the stability at −20 °C up to 6 months could be considered acceptable (>80%).

5.3. Comparison of whole blood and dried blood spots using authentic samples

Using the developed on-line LC–MS/MS method, investigations of real patient samples were performed. Both patient matrices, whole blood and DBS could be analyzed. In general, the determined concentrations correlated well between both methods. For morphine, benzoylecgonine and amphetamine, the more frequently illicit drugs detected in our laboratory in suspected cases of driving under the influence of drugs, correlation coefficients (r^2) are respectively, 0.981, 0.993 and 0.990 (Fig. 3).

6. Discussion

Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) is one of the methods of choice for the quantification of drugs and their metabolites in biological fluids. Following the new challenges encountered in the process of drugs determination, liquid chromatography–tandem mass spectrometry has been found to achieve high-throughput analysis with its column switching procedures. On-line sample cleaning methods, which allow the sample preparation and introduction steps to be combined into one, have been thoroughly investigated, including column switching. With this effective tool, the sample preparation step before analysis is simplified and the analytical process speeded up. The initial LC–MS/MS method developed in our laboratory for illicit drugs determination in plasma and serum required a deproteinization step before on-line extraction [2]. In our experience, we could observed a significant decrease of undesirable matrix effects with samples diluted in water before analysis, particularly in whole blood, dilution in water making easier protein-binding hydrolysis [1,2,20]. The persistent effects are more reproducible, with a signal to noise ratio lower as consequence, and are compensated using the deuterated analogues as internal standard. We believe that it would be of great interest to adapt the first described method with a sample step preparation reduced to a simple dilution of whole blood sample in water (1:50). The analytical validation of this adapted method allowed a comparison between fresh whole blood and DBS assays. In fact, it could be considered that a 3 mm diameter spot is equivalent to 3 μL of whole blood [3,6,7,14]; the

Table 4
Stability of compounds in DBS stored at 4 °C and −20 °C for 6 months.

Compound	4 °C		−20 °C	
	10 ng/mL[a] (%, $n=2$)	50 ng/mL[a] (%, $n=2$)	10 ng/mL[a] (%, $n=2$)	50 ng/mL[a] (%, $n=2$)
Morphine	64.2	65.8	91	85.3
Codeine	83	78.4	100	100
Cocaine	45.2	45.3	86.3	82
Ecgonine methylester	12.2	11.4	86	87.2
Benzoyl-ecgonine	100	100	100	94.1
Amphe-tamine	59.8	56	95.8	86.5
Metamphe-tamine	50.5	59.1	85.9	87.5
MDA	70.1	72.1	87.8	81.5
MDMA	69.8	75.2	84.3	88.5

[a] Stability was assessed in duplicate for each concentration and temperature; the results are expressed as % of initial concentration.

disk desorption being in 150 μL of water, the dilution is to 1:50, as for fresh whole blood. The validation assay showed a good correlation between analyses carried out in fresh whole blood samples versus in dried blood spots. The use of DBS technique coupled to on-line LC–MS/MS, in suspected cases of driving under the influence of drugs, represents a low-cost, rapid, precise and simple method, with a satisfactory low detection limit for only 30 μL of blood per spot (Fig. 2). The collection of capillary blood to be spotted on filter paper represents a less invasive and more feasible compared to venipuncture. DBS sampling allows not waiting for a medical doctor to collect venous blood, and can significantly reduce the time gap between suspicion of being under influence of drugs and sample collection, thus reflect the actual driving situation much better. Immediate drying of the collected blood spot can improve stability and reduce environmental influences. The water present in whole blood plays a role in hydrolysis reactions as an active reagent that cleaves molecules. Dehydration of the samples on the filtered paper minimizes the hydrolysis of drugs and results in enhancement of drug stability. The stability study indicated that the DBS storage for all molecules is possible up to 6 months, which is very interesting for suspected cases of driving under the influence of illicit drugs. For example, on the contrary of DBS, cocaine is unstable in whole blood because subjected to hydrolysis, as far as at 4 °C as at −20 °C. For two quantitative analysis of the same whole blood sample made in several weeks interval, results could be very different because of cocaine degradation. Moreover, the filter paper storage at −20 °C in a hermetic container protects DBS from humidity and moistures development. As far as matrix effects are concerned, ion suppression assays emphasized the absence of these undesirable effects associated with the use of paper card. As mentioned above, matrix effects decreased significantly with sample dilution of water. A dilution to 1:100 in fresh whole blood and DBS (3 mm diameter disk in 300 μL of water) was tested. A sufficiently sensitivity was obtained for all molecules. This dilution (1:100) allowed a second injection of 100 μL in the LC–MS/MS system to control the first result for example. It was decided to use 30 μL of fresh whole blood for DBS, its application assuring thorough filling of disk with the blood spot and the diameter is approximately the same as blood spots produced by patients. An analytical validation of an on-line LC–MS/MS quantification of cannabinoids in small whole blood sample (25 μL) is currently in progress. In parallel, is developed a cannabinoids quantitative analysis in dried blood spot, in order to process in the same DBS sample, the quantitative analysis of opiates, cocainics, amphetamines and cannabis, as required in suspected cases of driving under the influence of drugs. Owing to its chemical properties, delta-9-tetrahydrocannabinol (THC) and its metabolites require an extraction and chromatographic conditions specifics to them. The analysis of opiates, cocainics and amphetamines requiring only a 3 mm diameter disk, the remained part of the disk could be used for cannabinoids quantitative determination. An other DBS on-line LC–MS analysis was described in literature [21]. This method required an analytical system devoted to DBS analysis. The main advantage of our method is the possibility to use the on-line LC–MS/MS system, with the same mobile phase and analytical columns, as well for illicit drugs quantification in DBS, as for other drugs analysis practiced routinely in our laboratory in usual biological matrices, as plasma or urine.

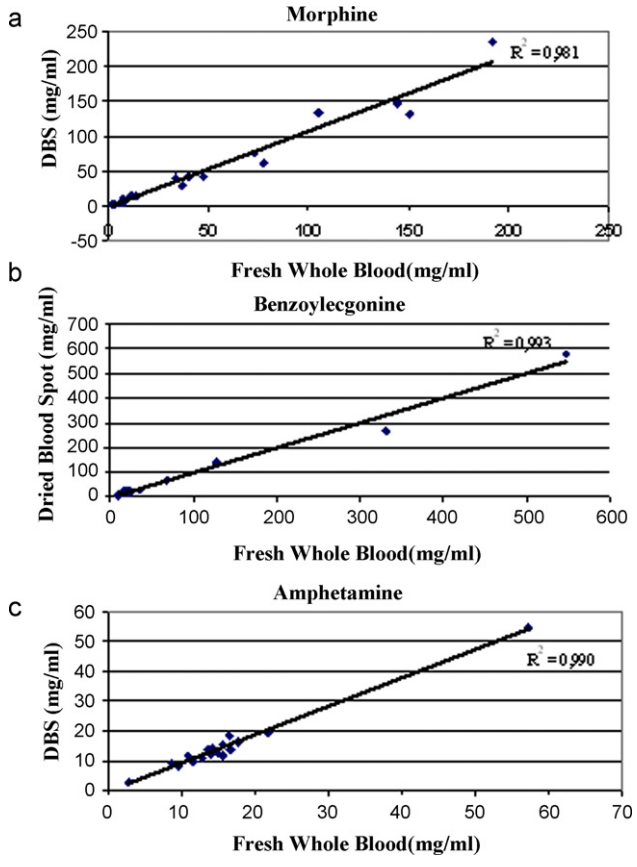

Fig. 3. Method comparison for morphine (a), benzoylecgonine (b) and amphetamine (c) between DBS and fresh whole blood assays.

7. Conclusion

A sensitive and specific on-line LC–MS/MS method used for the quantitative analysis of illicit drugs in small volumes of blood contained in dried blood spot has been developed. The DBS technique provides a suitable procedure for the analysis of sample in forensic toxicology because spots are easy to handle and facilitate samples

mailing as storage in the laboratory. DBS sampling has potential as precise and inexpensive option for illicit drugs (opiates, cocainics and amphetamines) quantitative analysis in suspected cases of driving under the influence of drugs.

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