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Simultaneous determination of cocaine and opiates in dried blood spots by electrospray ionization tandem mass spectrometry

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ABSTRACT

A sample pre-treatment method based on blood spot collection filter cards was optimized as a means of using small volume samples for the screening and confirmation of cocaine and opiates abuse. Dried blood spots (DBSs) were prepared by dispersing 20 μL of whole blood specimens previously mixed with the internal standards (deuterated analogs of each target), and subjecting the whole DBS to extraction with 5 mL of methanol under orbital-horizontal shaking (180 rpm) for 10 min. Determinations were based on direct electrospray ionization tandem mass spectrometry (ESI-MS/MS) by injecting the re-dissolved methanol extract with the delivery solution (acetonitrile–water–formic acid, 80:19.875:0.125) at a flow rate of 60 μL min^{-1}, and using multiple reaction monitoring (MRM) mode with the *m/z* (precursor ion)→*m/z* (product ion) transitions for acquisition. Matrix effect has been found to be statistically significant (Multiple Range Test) when assessing cocaine, BZE, codeine and morphine, and the use of the standard addition method (dispersion of whole blood previously mixed with standards onto the filter papers) was needed for accurate determinations. The developed DBS-ESI-MS/MS procedure offered good intra-day and inter-day precisions (lower than 10% and 12%, respectively), as well as good intra-day and inter-day accuracies (inter-day absolute recoveries, expressed as the mean analytical recovery over three target concentration levels, of 103%, 100%, 101%, 98% and 100% for cocaine, BZE, codeine, morphine and 6-MAM, respectively). The high sensitivity inherent to MS/MS determinations combined with the minimal dilution of sample allowed low limits of quantification for all targets, and the developed method results therefore adequate for cocaine and opiates screening and confirmation purposes. The procedure was finally applied to DBSs prepared from whole blood from polydrug abusers, and results were compared with those obtained after a conventional sample pretreatment method based on solid phase extraction for plasma specimens and gas chromatography–mass spectrometry.

1. Introduction

Since the first report by Guthrie and Susi in 1963 describing the collection of blood on absorbent papers for determining phenylalanine in the detection of phenylketonuria in newborns [1], the dried blood spot (DBS) technique has become the method of choice in clinical laboratories worldwide for the screening of several metabolic disorders in newborns [2]. DBS consists of blood diffusion onto standardized filter cards followed by an air-drying stage, and target extraction using a 3.2 mm diameter punch from each DBS. The main advantage of this simple method is the retention of the major blood components on the filter card, whereas targets can be extracted to a solution for a further analysis. The method is therefore simple, and clean extracts are commonly obtained. DBS in combination with tandem mass spectrometry is today the basis of the well established Expanded Newborn Screening (ENBS) methodologies for the simultaneous assay of various newborn diseases [3].

The small sample volume required (typically 20 μL) as well as the ease of shipping/storage, and analyte stability [4], are some of the advantages of the DBS based methods. As reviewed by Tanna and Lawson [5], other advantages of the DBS extraction process are the possibility of automation, and also the potential of this technique for direct analysis of the sample without prior extraction. Although

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plasma and serum can be loaded onto the filter cards, DBS is usually performed by dispersing whole blood. Methods based on whole blood are preferable in forensic-toxicological studies due to the difficulty of obtaining serum/plasma from whole blood in cases of death.

In addition to the wide application of DBS techniques in newborn screening [2], some developments describing the use of DBS for assessing therapeutic drugs have been reported [4,6]. These applications have focused on determining antibiotics [7,8], non-steroidal anti-inflammatory drugs [9], antidepressants [10], and various other pharmaceuticals compounds in blood [11–18]. The technique has also been applied for assessing benzoylecgonine (BZE), cocaine's main metabolite, in newborns and childbearing women [19–21]; and more recently, to assess morphine and 6-monoacetylmorphine (6-MAM) [22], and morphine and its active metabolites morphine 3β-glucuronide (M3G) and morphine 6β-glucuronide (M6G) [23]. In these developments, radioimmunoassay (RIA) has been used for screening purposes [19,20]; whereas, methods based on high performance liquid chromatography–mass spectrometry (HPLC–MS) [20] and high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) [21–23] have been used for confirmatory studies.

Reported DBS treatments consist of BZE extraction from a 1/4-inch punch with 200 μL of buffer containing phosphate-buffered saline (PBS) under continuous stirring for 18 h [19,20]. On other occasions, the 1/4-inch punches were pre-treated with 200 μL of 2 mM aqueous ammonium acetate overnight, followed by vortex mixing with 1 mL of methanol and centrifugation for removing the precipitated protein [21]. Similarly, morphine and metabolites are extracted from DBSs with 100 μL of HPLC grade water, followed by protein precipitation with 600 μL of methanol/0.2 M $ZnSO_4$ (7:3, v/v) [23].

The objective of the current work has been the development of a DBS methodology as a simple and low cost sample pre-treatment method for the simultaneous isolation of cocaine, BZE, morphine, codeine and 6-MAM from whole blood. Efforts have been made for performing fast and efficient target isolation from DBSs. Different organic solvents (methanol, acetonitrile and methyl tert-butyl ether) were directly tested as extractants for achieving selective target extraction and avoiding protein solubilization from the DBSs. Direct electrospray ionization (ESI) tandem mass spectrometry (MS/MS) has been proposed in the current application for both screening and confirmatory studies. As previously mentioned, direct tandem MS/MS determinations are commonly performed in newborn screening trials after DBS [2,3]. Some direct MS/MS developments have also been reported when assessing therapeutic drugs in serum [24,25], amphetamines, opiates, cannabinoids and benzodiazepines in urine [26], and cocaine, opiates and metabolites in hair [27] after efficient target isolation procedures. The short acquisition time when performing direct MS/MS measurements (approximately 90 s for each run) leads to fast determinations, which allows for increasing the number of samples to be screened/confirmed per time unit, and reduces hence the time needed by the toxicological-forensic laboratory.

2. Experimental section

2.1. Apparatus

An API 2000 LC/MS/MS system equipped with a Perkin Elmer Series 200 chromatographic pump and a Perkin Elmer Series 200 autosampler (PE Sciex, Concord, Canada) was used for direct ESI-MS/MS determinations. A Hewlett Packard Model 6890 gas chromatograph (Hewlett-Packard, Avondale, PA, USA), equipped with a HP-5 capillary column (30 m, 0.22 mm I.D., 0.33 μm film thickness of cross-linked 5% phenyl methyl silicone) and a HP 5973 mass spectrometer was used for GC–MS analysis. DBSs were prepared and allowed to air-dry in a Class-100 clean fume hood (Telstar S.A., Terrassa, Spain). A Boxcult incubator situated on a Rotabit orbital-rocking platform shaker from J.P. Selecta (Barcelona, Spain) was used for performing targets extraction from DBSs. Other laboratory devices were: a Visiprep TM DL vacuum manifold from Supelco (Bellefonte, USA); Univeba and Digiterm 3000542 thermostatic baths (Selecta, Barcelona, Spain); an Orion 720A plus pH-meter with a glass–calomel electrode (Orion, Cambridge, UK); a Reax 2000 mechanical stirrer (Heidolph, Kelheim, Germany); and a VLM EC1 metal block thermostat and N_2 sample concentrator from VLM (Leopoldshöhe-Greste, Germany).

2.2. Reagents and materials

Ultrapure water (resistivity, 18 MΩ cm) was obtained from a Milli-Q purification device (Millipore Co., Bedford, MA, USA). Drug stock standard solutions were prepared from cocaine (1000 mg L^{-1} dissolved in acetonitrile), BZE (1000 mg L^{-1} dissolved in methanol), codeine (1000 mg L^{-1} dissolved in methanol), morphine (1000 mg L^{-1} dissolved in methanol) and 6-MAM (1000 mg L^{-1} dissolved in acetonitrile) from Lipomed (Arlesheim, Switzerland). Deuterated drug stock standard solutions were prepared from cocaine-d_3 in acetonitrile (100 mg L^{-1}), BZE-d_3 in methanol (100 mg L^{-1}), codeine-d_3 in methanol (100 mg L^{-1}), morphine-d_3 in methanol (100 mg L^{-1}), and 6-MAM-d_3 in acetonitrile (100 mg L^{-1}) from Cerillant (Round Rock, TX, USA). The filter cards used for DBSs preparation were Whatman 2012-10 from Whatman (Dassel, Germany). Bond Elut Certify cartridges (130 mg sorbent weight, 3 mL volume) used for performing SPE were obtained from Varian (Lake Forest, CA, USA). Acetonitrile (supragradient HPLC grade) was from Scharlau (Barcelona, Spain). Methanol (HPLC grade) and formic acid (98–100%) were from Merck (Poole, U.K.). Other reagents were: methyl tert-butyl ether, hydrochloric acid 37% (m/m) and phosphoric acid 85% (m/m) from Panreac (Barcelona, Spain); ammonium hydroxide, chlorotrimethylsilane (TMCS) and N-methyl-tert-butylsilyltrifluoroacetamide (BSTFA), potassium dihydrogen phosphate, potassium dihydrogen phosphate, and disodium hydrogen phosphate from Merck.

Contamination was minimized after subjecting glassware and plastic ware to a washing procedure with a diluted soap solution, thoroughly rinsed with tap water, then three times with ultra-pure water, and finally kept in a bleach solution for 24 h. After this treatment, the ware was rinsed several times with ultra-pure water before use.

2.3. Whole blood samples.

Whole blood and plasma samples used in the current study were from polydrug abusers under control in an addiction research center in Santiago de Compostela. Drug-free whole blood samples used for method validation were obtained from the General Laboratory (Complexo Hospitalario Universitary de Santiago, CHUS) in Santiago de Compostela. For all cases, whole blood samples were kept at 4 °C, if necessary.

2.4. Dried blood spot sample preparation

1.5 cm × 1.5 cm pieces were previously cut from Whatman 2012-10 filter cards, and 20 μL of samples/solutions were dispersed into the center of the 2.25 cm^2 square pieces. Spotted samples (20 μL) consisted of whole blood (140 μL) mixed with 10 μL of a solution containing the internal standards (cocaine-d_3 and BZE-d_3 at 5 μg mL^{-1}, and morphine-d_3, codeine-d_3 and 6-MAM-d_3 at 25 μg mL^{-1}) and 100 μL of ultrapure water (final volume of 250 μL). When performing experiments with spiked whole blood samples (method validation), different volumes (0, 5, 25, 50 and 100 μL) of a mixture of standards (1 μg mL^{-1} of cocaine