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Identification and quantification of psychoactive drugs in whole blood using dried blood spot (DBS) by ultra-performance liquid chromatography tandem mass spectrometry

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ABSTRACT

A procedure based on ultra-high-pressure liquid chromatography tandem mass spectrometry has been developed for the determination of twenty three psychoactive drugs and metabolites in whole blood using dried blood spot (DBS). Chromatographic separation was achieved at ambient temperature using a reverse-phase column and a linear gradient elution with two solvents: 0.1% formic acid in acetonitrile and 5 mM ammonium formate at pH 3. The mass spectrometer was operated in positive ion mode, using multiple reaction monitoring via positive electro-spray ionization. The method was linear from the limit of quantification (5 ng/ml for all the analytes apart from 15 ng/ml for Δ-9-tetrahydrocannabinol and metabolites) to 500 ng/ml, and showed good correlation coefficients ($r^2 = 0.990$) for all substances. Analytical recovery of analytes under investigation was always higher than 75% and intra-assay and inter-assay precision and accuracy always better than 15%.

Using the validated method, ten DBS samples, collected at the hospital emergency department in cases of acute drug intoxication, were found positive to one or more psychoactive drugs. Our data support the potential of DBS sampling for non invasive monitoring of exposure/intoxication to psychoactive drugs.

1. Introduction

Psychoactive drugs are substances with the ability to act on normal brain mechanisms and change an individual's consciousness, mood or thinking processes. Many psychoactive drugs are legal and used as medications (i.e. benzodiazepines, antidepressant and sedatives), others, illegal, for recreational purpose (i.e. opiates, cannabis, hallucinogens, cocaine) [1]. The detection of psychoactive drugs in biological conventional (urine and blood) and non conventional (e.g. oral fluid, hair) matrices is of great importance in clinical and forensic toxicology such as workplace drug testing, roadside testing, therapeutic drug monitoring, rehabilitation programs and post-mortem cases. [2].

Blood is the specimen of choice to detect and quantify a drug if there is suspicion of current drug-related impairment or intoxication. However, its collection requires skilled professional figures, patients can undergo discomfort during collection, which can be not risk-free. The dried blood spot (DBS) use allows the collection of blood samples from a small finger prick with an automatic lancet and offers many benefits in comparison with conventional venipuncture: simple and non-invasive collection which can be executed by non skilled personnel; no need of anticoagulant or plasma separation, easy sample shipment and storage [3]. Most of the pathogenic agents, which can be present in blood, are deactivated on the filter paper [3].

Dried blood spots (DBS) technique was firstly developed in 1963 to be used in neonatal screening for phenylketonuria and for both

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qualitative and quantitative screening of metabolic disorders [4], but in the more recent years DBS has gained interest as an alternative haematic matrix [3,5,6] for therapeutic drug monitoring [7], pharmaco-toxicokinetic studies [8] and for drug testing in sports [9].

Several publications suggest also the potential of DBS for detecting exposure to psychoactive drugs. Analytes measured include both legal drugs (scheduled drugs available on prescription) and psychoactive illicit drugs including benzodiazepines, amphetamines type substances, cocaine, cannabinoids, opiates, gamma-hydroxybutyric acid (GHB) and ethylglucuronide (EtG) [10–23].

A substantial number of these reports describe DBS application for only one class of drugs and their respective metabolites [11–13,15–17,19,20,23] and the vast majority of available publications describe the analysis of DBS obtained by pipetting patients blood samples onto the card [11,12,17–19,22].

We here present the development and validation of an ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS) method for the simultaneous determination of a panel of psychoactive drugs in DBS. The method has been applied in real cases of individuals admitted to the hospital emergency rooms for acute intoxication related to the consumption of principal psychoactive drugs.

2. Experimental

2.1. Chemicals and reagents

Morphine, codeine, 6-monoacetylmorphine (6-MAM), methadone, EDDP, cocaine, benzoylecgonine, Δ-9-tetrahydrocannabinol (THC), 11-hydroxy-Δ-9-tetrahydrocannabinol (THC-OH), 11-nor-9-carboxy-Δ-9-tetrahydrocannnabinol (THC-COOH), amphetamine, methamphetamine, 3,-4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-*N*-propyl-amphetamine (MDPA) and nalorphine used as internal standards (ISs) were supplied by LGC Standards (Milan, Italy).

Alprazolam, alprazolam-d_5 used as internal standard (IS), clonazepam, diazepam, diazepam-d_5 (as IS), nor-diazepam, lorazepam, lorazepam-d_4 (as IS), lormetazepam, medazepam, oxazepam, oxazepam-d_5 (as IS) and midazolam were obtained from Cerilliant (Austin, TX, USA).

Ultrapure water and all other reagents of UHPLC–MS/MS grade were obtained form Sigma-Aldrich (Milan, Italy).

Whatman (Maidstone, UK) 903 Protein Saver cards were used for DBS collection.

2.2. DBS samples

Ten individuals admitted to the Emergency Department of Hospital del Mar, Barcelona, Spain with symptoms of psychoactive drug intoxication underwent a clinical examination and symptomatic treatment to overcome the intoxication effects (e.g. unconsciousness, agitation, high pressure, sweating, slurred speech, urinary incontinence). Urine samples were collected and immediately analysed for eventual presence of drugs of abuse by immunoassay. At the same time, DBSs were collected by patients finger prick on a pre-marked card (a blood drop filling a pre-marked circle corresponded to approximately 30 μL blood) and stored at ambient temperature. The study was approved by the Institutional Ethical Committee (IMIM-IMAS Hospital del Mar Ethical Committee) and conducted in accordance with the Declaration of Helsinki. Signed informed consent was obtained from each individual.

2.3. Calibration standards and quality control samples

Stock standard solutions (1 mg/ml) of each analyte were prepared in methyl alcohol and stored at −20 °C. From stock solutions, working solutions of 10, 1 and 0.1 μg/ml of the analytes under investigation were made in water and used for the preparation of calibration curves and quality control (QC) samples. ISs were diluted in water to give a working solution at a concentration of 1 μg/ml and stored at −20 °C.

Calibration standards containing 5, 10, 50, 100, 200 and 500 ng of analytes under investigation per ml of whole blood were prepared by adding suitable amounts of water working solutions to 1 mL of pre-checked whole blood pool samples. In case of Δ-9-tetrahydrocannabinol and metabolites, the first point of calibration curve was set at 15 ng/ml (0.45 ng/DBS). Then, by using a glass microsyringe, 30 μL blood was applied to the card, filling the pre-marked circle (calibration ranges: 0.15, 0.3, 1.5, 3 and 15 ng/DBS). QC samples of 6 (0.18) and 20 (0.6) in the case of Δ-9-tetrahydrocannabinol and metabolites (low control), 150 (4.5) (medium control) and 400 (12) (high control) ng analytes/ml (ng analytes/BDS) whole blood and samples at the limit of quantification (LOQ) were also prepared as above reported for the calibration standards. QC samples were included in each analytical batch to check method linearity, intra- and inter- assay inaccuracy and imprecision, matrix effects, recovery and process efficiency.

2.4. DBS preparation

Pre-marked cards spotted with 30 μL blank blood, calibrators, QC and real samples were cut, once dried, at the edges and placed into a tube with 10 μL ISs working solution (1 μg/ml) and 990 μL methyl alcohol. The tube was then sonicated for 15 min and centrifuged at 3500*g* for 5 min. The supernatant was brought to dryness under vacuum, re-dissolved with 100 μL UHPLC mobile phase (solvents A/B, 80/20, v/v) and 10 μL injected into the UHPLC–MS/MS system.

2.5. Ultra-high-pressure liquid chromatography tandem mass spectrometry (UHPLC–MS/MS)

Analytes under investigation in DBS were detected using an ultra-high performance liquid chromatography system (Waters Acquity UPLC, Waters Corporation, Milan, Italy) coupled with a triple quadrupole mass spectrometer (Waters Xevo TQ, Waters Corporation). Chromatography was carried out in reversed phase Acquity UPLC HSS C18 column (2.1 mm × 150 mm, 1.8 μm, Waters Acquity UPLC, Waters Corporation, Milan, Italy) using a linear gradient elution with two solvents: 0.1% formic acid in acetonitrile (solvent A) and 5 mM ammonium formate pH 3.0 (solvent B). Solvent A was maintained at 5% for the first 0.50 min. It was increased to 55% from 0.50 to 10.00 min, then increased to 90% from 10.00 to 10.75 min, held at 90% from 10.75 to 12.85 min, and then decreased back to 5% from 12.85 to 13.00 min and held at 5% from 13.00 to 16.50 min for re-equilibration. The flow rate was kept constant at 0.40 mL/min during the analysis. The separated analytes were detected with a triple quadrupole mass spectrometer operated in multiple reaction monitoring (MRM) mode via positive electrospray ionization (ESI). The applied ESI conditions were the following: capillary voltage 1.3 kV, desolvation temperature 600C, source temperature 150C, cone gas flow rate 20 L/h, desolvation gas flow rate 1000 L/h and collision gas flow rate 0.13 mL/min. Cone energy voltages, MRM transitions, and collision energy voltages were established for each analyte and the values are listed in Table 1.