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Sensitive screening of abused drugs in dried blood samples using ultra-high-performance liquid chromatography-ion booster-quadrupole time-of-flight mass spectrometry

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ABSTRACT

An increased rate of drug abuse is a major social problem worldwide. The dried blood spot (DBS) sampling technique offers many advantages over using urine or whole blood sampling techniques. This study developed a simple and efficient ultra-high-performance liquid chromatography-ion booster-quadrupole time-of-flight mass spectrometry (UHPLC-IB-QTOF-MS) method for the analysis of abused drugs and their metabolites using DBS. Fifty-seven compounds covering the most commonly abused drugs, including amphetamines, opioids, cocaine, benzodiazepines, barbiturates, and many other new and emerging abused drugs, were selected as the target analytes of this study. An 80% acetonitrile solvent with a 5-min extraction by Geno grinder was used for sample extraction. A Poroshell column was used to provide efficient separation, and under optimal conditions, the analytical times were 15 and 5 min in positive and negative ionization modes, respectively. Ionization parameters of both electrospray ionization source and ion booster (IB) source containing an extra heated zone were optimized to achieve the best ionization efficiency of the investigated abused drugs. In spite of their structural diversity, most of the abused drugs showed an enhanced mass response with the high temperature ionization from an extra heated zone of IB source. Compared to electrospray ionization, the ion booster (IB) greatly improved the detection sensitivity for 86% of the analytes by 1.5–14-fold and allowed the developed method to detect trace amounts of compounds on the DBS cards. The validation results showed that the coefficients of variation of intra-day and inter-day precision in terms of the signal intensity were lower than 19.65%. The extraction recovery of all analytes was between 67.21 and 115.14%. The limits of detection of all analytes were between 0.2 and 35.7 ng mL^{-1}. The stability study indicated that 7% of compounds showed poor stability (below 50%) on the DBS cards after 6 months of storage at room temperature and −80 °C. The reported method provides a new direction for abused drug screening using DBS.

1. Introduction

Drug abuse is an increasing global social burden. Most social problems are associated with drug abuse, including sexual assault, child abuse, suicide, murder, traffic accidents, and violence. The increase of drug abuse rates is a global problem. Globally, the occurrence of drug abuse including opioid, cocaine and amphetamine drugs was 5.2% (range: 3.5–7.0%) in the 15–64-year-old population from 2009 to 2012 [1]. In 2013, the estimated rate of drug abuse including cocaine, hallucinogens, and nonmedical use of prescription-type sedatives in the United States was 9.4% of the population aged 12 or older [2]. Therefore, it is important to have efficient and effective screening methods for drug-related crime and clinical management.

Several analytical methods have been developed to analyze abused drugs in biological samples include urine and whole blood [3–7]. Currently, urine is the most commonly used biological fluid

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for drug testing. However, adulteration of urine samples may give false positive results, and it is not possible to obtain urine samples in the case of death. Although blood samples avoid certain limitations of urine samples, the invasive collection and the storage and handling of whole blood samples limit the wide application of this sampling method. Additionally, blood collection may cause the transmission of some diseases such as HIV, hepatitis, and other blood-borne viruses [8].

The dried blood spot (DBS) sampling technique has a long history that can be traced back to 1960, but until recently this technique had not gained great attention in the bioanalysis of adults. The DBS method spots a small amount of blood on a filter paper card followed by a drying process. The drying process increases the stability of most photosensitive compounds [9,10]. Additional advantages of the DBS technique include low biohazard risk during the shipment of samples and decreased invasiveness [11–13]. Although the DBS technique is increasingly used in medical care due to its broad advantages, the application of this convenient sampling technique to abused drugs is still rare.

To date, only a few methods have been developed to analyze abused drugs in DBS. Radioimmunoassays using DBS samples have been used to analyze benzoylecgonine (BZE), a main metabolite of cocaine, in newborns and child-bearing women [14,15]. Cocaine and its metabolites have been analyzed in DBS by high-performance liquid chromatography fluorescence [16]. One of the main drawbacks of the DBS technique is its sensitivity because only a small amount of blood is spotted on the card. The high sensitivity and selectivity of liquid chromatography-electrospray ionization mass spectrometry (LC–ESI–MS) makes the technique a perfect match for coupling with DBS analysis. Therefore, growing numbers of LC–MS methods have been developed to analyze abused drugs on DBS cards. However, most studies have only included very limited abused drugs such as cocaine, opiates and benzodiazepines [17–19]. Recently, Odoardi et al. developed an LC–MS/MS method to analyze opiates, methadone, fentanyl and analogues, cocaine, amphetamines and amphetamine-like substances, but the sample extraction time was not efficient, which may hamper its application to routine screening work[20].

Currently, the most commonly used MS platforms to analyze drugs of abuse in biological samples are triple quadrupole and time-of-flight (TOF) mass spectrometry [4,21–25]. The need for pre-established transition ion pairs and the decreased sensitivity with the increased number of transition ion pairs in multiple reaction monitoring mode are the major limitations of triple quadrupole mass spectrometry [26]. Time-of-flight mass spectrometry provides accurate mass measurement at the millidalton (mDA) range as well as high full-scan sensitivity. The high mass accuracy provides the advantage of using exact monoisotopic masses and isotopic patterns for compound identification. This advantage provides an opportunity for extension of the screening targets. However, with respect to the identification of unknown abused drugs in biological samples, TOF-MS can give false positive results when simply identifying by accurate mass [27]. Therefore, creating spectral information using a hybrid mass such as quadrupole TOF-MS (QTOF-MS) is an effective method for the identification and confirmation of abused drugs. Recently, methods using QTOF-MS have been developed for the screening and confirmation of abused drugs in biological samples [26,28]. Considering the selectivity and sensitivity of LC–QTOF–MS, using this powerful tool will be beneficial for abused drug screening on DBS samples.

This study aims to develop a simple and efficient analytical method to screen a wide range of abused drugs in DBS samples using UHPLC-IB-QTOF-MS. The most commonly used abused drugs, including amphetamines, opioids, cocaine, benzodiazepines, ketamine, lysergic acid diethylamide (LSD) and many other new and emerging abused drugs were selected as our target analytes. Metabolites including norketamine, norephedrine, 7-aminoflunitrazepam, and nordiazepam were also included in this study. To provide sufficient sensitivity for low-concentration drugs in the small quantities of DBS samples, an ion booster (IB) ion source with an extra heated spray zone was applied. IB uses a controlled vaporizer temperature to enhance ionization efficiency of the target analytes by evaporating the solvent of analyte ions even at high mobile phase flow rates. As the sensitivity improvement using IB source depends upon the chemical properties and thermal stability of the analytes, this study evaluated the sensitivity improvement for various abused drugs. In addition, this study performed a six-month stability test to investigate the stability of abused drugs on the card. Although improving compound stability is one of the main advantages of the DBS sampling technique, there is no previous research investigating the stability of abused drugs on DBS cards, which limits its use in real-world analysis. To the best of our knowledge, this is the first study developing a simple and efficient UHPLC-IB-QTOF-MS method for a wide range of abused drugs using a DBS sampling technique. This method offers a new approach for abused drug control.

2. Experimental

2.1. Standards and reagents

Amphetamine, alprazolam, 7-aminoflunitrazepam, amobarbital, aminorex, bromazepam, butalbital, butorphanol, 4-bromo-2,5-dimethoxyphenethylamine (2C-B), butabarbital, clonazepam, chlordiazepoxide, clobazam, dihydrocodeine, diazepam, ephedrine, estazolam, fentanyl, flurazepam, flunitrazepam (FM2), ketamine, lorazepam, lormetazepam, LSD, methamphetamine, 4-methoxyamphetamine (PMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxy-N-ethylamphetamine (MDEA), *para*-methoxymethamphetamine (PMMA), meperidine, methadone, midazolam, methylephedrine, methylphenidate, norketamine, norephedrine, nitrazepam, nordiazepam, nalorphine, lorazepam, pentazocine, phentermine, prazepam, pseudoephedrine, secobarbital, triazolam, temazepam, tramadol, and zolpidem were purchased from Cerilliant (Round Rock, Texas, USA). Cocaine hydrochloride, codeine, morphine, pentobarbital, barbital, phenobarbital, and ammonium acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phencyclidine (PCP) was purchased from Triage® Tox Drug Screen, Biosite, (San Diego, CA, USA). Acetonitrile (ACN) and acetic acid were purchased from Merck (Darmstadt, Germany). MeOH and DI water were purchased from Scharlau (Spain). Ammonium bicarbonate was purchased from J.T. Baker (Phillipsburg, NJ, USA). All reagents and solvents used were of LC–MS grade.

2.2. UHPLC-IB-QTOF-MS

The Agilent 1290 UHPLC system consisted of a degasser and a quaternary solvent pump (Agilent Technologies, Santa Clara, CA) coupled with a Bruker maXis QTOF (Bruker, Rheinstetten, Germany) equipped with an IB source. A Poroshell EC-C18 column (2.1 × 100 mm, 2.7 μm, Agilent) was used for compound separation. The mobile phase was composed of 0.1% acetic acid in DI water (solvent A) and MeOH (solvent B). The gradient profile used for positive ionization detection was as follows: 0–5 min, 2–20% B; 5–9 min, 20–60% B; 9–13 min, 60–90% B; 13–15 min 90–95% B; and then re-equilibration of the column for 3 min. The gradient for negative ionization detection was as follows: 0–2.5 min, 25–65% B; 2.5–4 min, 65–85% B; 4–5 min, 85% B. The column re-equilibration