Chapter 47

Assays for MDMA and Its Metabolites

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Abbreviations

CE Capillary electrophoresis
FL Fluorescent
GC Gas chromatography
HHMA 3,4-Dihydroxymethamphetamine
HMA 4-Hydroxy-3-methoxyamphetamine
HMMA 4-Hydroxy-3-methoxymethamphetamine
HPLC High-performance liquid chromatography
MDA 3,4-Methylenedioxyamphetamine
MDMA 3,4-Methylenedioxymethamphetamine
MS Mass spectroscopy
UV Ultraviolet

INTRODUCTION

3,4-methylenedioxymethamphetamine (MDMA), commonly known by the street name ecstasy, is classified as an illicit drug in most parts of the world. This compound is used recreationally to increase the sense of well-being (Es'haghi, Mohtaji, Hasanzade-Meidani, & Masrournia, 2010). The identification and quantification of this compound in biological and seized samples has a crucial role in the fields of toxicology, pharmacology, forensic science, and the profiling of seized samples.

Various analytical methods have been introduced to identify and quantify MDMA and its metabolites, such as 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxyamphetamine (HMA), 3,4-dihydroxymethamphetamine (HHMA) and 4-hydroxy-3-methoxymethamphetamine (HMMA) (Figure 1). This chapter describes the qualitative and quantitative methods that are conducted with different analytical instruments, such as high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), and gas chromatography (GC), which are coupled with diverse detection techniques, such as mass (MS) spectroscopy, fluorescent (FL) detection, and ultraviolet (UV) detections.

MATRICES

In forensic and toxicology sciences, biological samples such as urine, blood, hair, oral fluid (OF), sweat, nails, or any other tissues are the common matrices. In the profiling process, the common matrices are powders and tablets. Drug profiling is used to obtained information about chirality and related substances of seized samples of MDMA. These data help to identify the precursors and synthesis pathways, as well as to distinguish the samples' origins and manufacturers. In drug profiling, the sampling process and sample preparation is not a sophisticated procedure. However, if the analyst uses biological samples, the sampling and sample preparation becomes a problematic process and a rate-limiting step in the determination of MDMA and its metabolites.

Blood and urine are the two most common and conventional matrices. Blood samples need extensive sample preparations and cleanup processes, due to the presence of exogenous and endogenous compounds, metabolites, proteins, blood cells, and other constituents. In the case of urine samples, due to the filtration properties of the kidneys, this issue is less significant. Blood and urine samples are important to study the pharmacokinetic and metabolism of MDMA. About 20% of routine ingested dose of MDMA is excreted unchanged in the urine and the remaining of ingested dose is metabolized by the liver (da Silva et al., 2010). Due to autoinhibitory effect of MDMA on its own metabolism, the extent of hepatic metabolism is varying and depends on ingested dose (Van et al., 2007). MDMA and its metabolites were determined in these matrices with different sample preparation techniques and analytical methods (Bogusz, Kala, & Maier, 1997; Concheiro et al., 2007; Laloup et al., 2005; Michel, Rege, & George, 1993; Pichini et al., 2008; Shima et al., 2007; da Silva et al., 2010).

OF is considered a main alternative to a blood sample (Concheiro, de Castro, Quintela, Lopez-Rivadulla, & Cruz, 2005; Laloup et al., 2005). This sample contains salivary gland secretions and substances which are presented in the oral cavity (Concheiro et al., 2005). OF has several advantages over conventional matrices: first, its collection is noninvasive and can be done under direct supervision to prevent sample adulteration, substitution, and dilution. Second, it can be collected by simple expectoration or using a marketed OF collection device. Third, compounds are typically detectable 12–24 h after consumption in OF, which is sometimes a shorter period than in urine (Concheiro et al., 2005; Laloup et al., 2005). For these reasons, OF has become an important sample in the field of clinical and forensic toxicology in recent

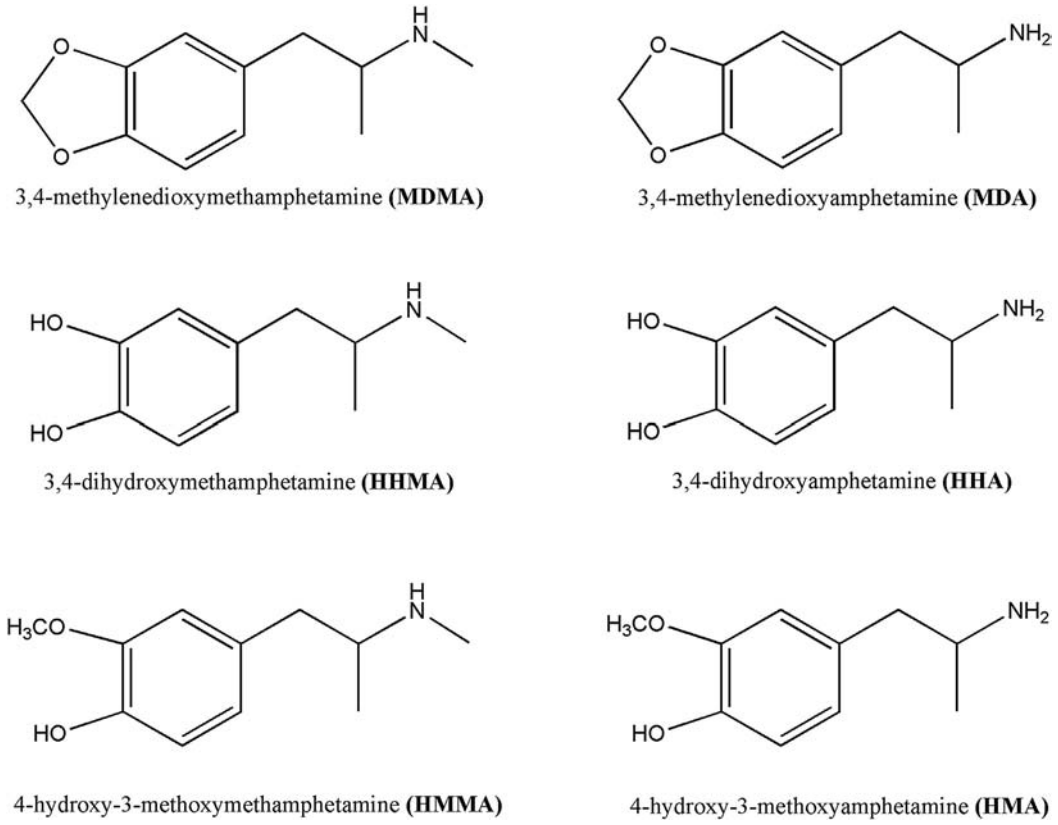

FIGURE 1 **Chemical structures of MDMA and its metabolites.**

years (Ventura et al., 2008). However, this matrix experiences some disadvantages and limitations. An important factor that can affect OF testing is the OF secretion, which may be stimulated during sample collection. OF secretion can be stimulated either mechanically (chewing on Parafilm or chewing gum) or by an acidic stimulant, such as acidic candy. By increasing the secretion of saliva in the oral cavity, drug concentrations are reduced due to dilution. Additionally, decreased salivary secretion (due to a side effect of many drugs, including sympathomimetics) results in a reduced volume of OF available for analysis. Drug concentrations in OF can also be affected either by OF pH fluctuations or by adsorption to the collection devices (Concheiro et al., 2005; Scheidweiler & Huestis, 2006). Metabolite concentrations are often much lower than parent drug concentration. Thus, analytical sensitivity is another concern when conducting OF drug testing (Klys, Rojek, Wozniak, & Rzepecka-Wozniak, 2007; Scheidweiler & Huestis, 2006). Moreover, there is no relation between concentrations of drugs in OFs and administered doses. MDMA and MDA were determined in OF by different analytical methods (Laloup et al., 2005). Concheiro et al. (2005) reported that MDMA and MDA were degraded independent of temperature in water and OF (except for MDA in OF, which is also temperature-dependent). Some reports indicate MDMA and MDA are stable in deionized water (Jamali, Ardakani, Rouini, et al., 2013), serum, and urine (Clauwaert, Van Bocxlaer, & De Leenheer, 2001) at temperatures of −20, 4, and 20 °C.

Another matrix in biological samples is sweat. Sweat testing is similar to OF in that it is noninvasive, offers reduced opportunities for sample adulteration, and sometimes provides longer detection time than plasma or urine. Disadvantages of sweat as an alternative specimen include a lack of information about dose–response relationships, lower analyte concentrations, and the need for a sensitive laboratory analysis system (De Martinis, Barnes, Scheidweiler, & Huestis, 2007; Pacifici et al., 2001). It was reported MDMA can be detected in sweat 2 h after ingestion of 100 mg MDMA and last as long as 12 h after MDMA ingestion (Pacifici et al., 2001). In another study, the reported amount of MDMA and MDA, 5 h after administration of 1 mg/kg of MDMA, in sweat was 629 and 21 ng/patch, respectively. HMMA and HMA concentrations were lower than the detection limit of analytical methods (5 ng/patch) (De Martinis et al., 2007).

Keratinized matrices such as hair and nails have been gaining attention in the fields of toxicology and forensic sciences (Kim, Cheong, Kim, Lee, & In, 2008; Kim, Shin, & In, 2010; Wada et al., 2012). These samples provide objective information regarding a patient's illicit drug habit over months. They can be used to determine whether or not an infant was exposed to illicit drugs in utero.

The rate of fingernail growth has been reported to be approximately about 0.1 mm/day (Kim et al., 2010). The collection of this sample is simple and noninvasive. Furthermore, the parent drug and its metabolite are accumulated inside the matrix; they are stable for long periods of time and are difficult to modify. It has been shown that the axial distribution of a drug in the nail provides a wide window of time for detection that cannot be obtained with traditional matrices such as blood or urine. MDMA and its metabolites were determined in fingernails with MS-based analytical techniques (Kim et al., 2008, 2010).

Hair is another keratinized structure in the body. Since ingested drugs are deposited in the hair root and shaft, detection of illicit drugs in hair is an efficient, robust, and rapid procedure (Aleksa et al., 2012). Normal hair growth rates are about 1.27 cm per month (Es'haghi et al., 2010), but it depends on the environmental temperature, position of the hair on the body, and the individual's race. Hair contains a relatively high ratio of parent drug to metabolite, which means that it is easy to identify specific biomarkers. Furthermore, hair gives the additional advantages that it can be easily obtained, not easily adulterated, and stored and transported without specific precautions due to its stability. Hair samples have applications in evaluating environmental exposure to toxicants from the intrauterine period of life, in doping control, and in drug abuse studies. However, this matrix has some pitfalls, such as the complexity of the matrix, external hair contamination, hair cosmetic treatments such as dyeing, bleaching, or permanent waving, racial differences, and irregular speed of hair growth. These pitfalls can complicate the interpretation of hair testing results (Lee et al., 2012). Knowledge of such pitfalls is crucial to avoiding wrong results and wrong interpretations of correct results. Another limitation in this matrix is the lower drug concentration and sample size compared with urine or blood (Es'haghi et al., 2010), which means that the hair sample analysis needs more sensitive analytical methods (Wu, Lin, Chen, & Chang, 2008). MDMA and its metabolites were determined in hair with different sample preparation techniques and analytical methods (Es'haghi et al., 2010; Klys et al., 2007; Martins, Yegles, Samyn, Ramaekers, & Wennig, 2007; Wada et al., 2012).

Ecstasy and its metabolites were also determined in other matrices such as perfusion medium (Jamali, Ardakani, Foroumadi, Kobarfard, & Rouini, 2013) and vitreous humor (Clauwaert et al., 2000). Perfusion medium is used when the organs of animals are isolated from the systemic blood circulation to study the pharmacokinetics and pharmacodynamics of a specific compound on that organ (Jamali, Ardakani, Foroumadi, et al., 2013), and vitreous humor is used when dealing with severely burned or decomposed bodies (Clauwaert et al., 2000).

Fingerprints are an interesting and strange matrix which was introduced in 2009. Szynkowska et al. reported that if someone touches powdered MDMA, a printed fingerprint contained MDMA, even if the person gently cleans the contaminated fingers with a soft cloth. This tiny amount of MDMA in a printed fingerprint can be measured by the time-of-flight secondary ion MS method (Szynkowska, Czerski, Rogowski, Paryjczak, & Parczewski, 2010). Banknotes are another irregular matrix which is used for the determination of MDMA and MDA (Wimmer & Schneider, 2011).

Beside the different matrices mentioned, the methods of sampling can also show discrepancies. Microdialysis is a novel method of sampling. In this method, the analytes are recovered by the use of a semipermeable membrane. Microdialysis permits long-term sampling with minimal damage to organs and no need to perform a sample cleanup procedure. Tomita, Nakashima, Wada, and Nakashima (2007) used this sampling method for obtaining rat blood and brain samples which contained MDMA and MDA.

SAMPLE PREPARATION

Sample preparation is used to clean up and eliminate the interfering compounds in the matrices. This process is time-consuming, and in some cases, it is the rate-limiting step in the determination procedure. Besides these limitations, this process can concentrate the analytes in the matrices and increase the selectivity and specificity of analytical methods. For these reasons, the analytical methods with simple or without any sample preparation techniques are preferred. Among sample preparation technique, precipitation, liquid–liquid extraction (LLE), and solid-phase extraction (SPE) are the most commonly used techniques in the analysis of illegal drugs. Precipitation of urine samples with methanol was reported for the determination of MDMA, MDA, and HHMA as free form and conjugated with glucuronic acid and sulfate (Shima et al., 2007). Diethyl ether (Buechler et al., 2003; Mueller, Yuan, et al., 2009), ethyl acetate (Shima et al., 2007), *n*-hexane:ethyl acetate (7:3 by volume) (Clauwaert et al., 2000; da Costa & da Matta Chasin, 2004), *n*-hexane:ethyl acetate (2:1 by volume) (Cheze, Deveaux, Martin, Lhermitte, & Pepin, 2007), *n*-hexane (Wada, Nakamura, Tomita, Nakashima, & Nakashima, 2005), or ethyl acetate:*n*-hexane:1-butanol (10:10:1 by volume) (Michel et al., 1993) were used as LLE solvents for the extraction of MDMA and its metabolites from matrices such as serum, blood, vitreous humor, and urine. Moreover, LLE by "Toxitube A" was reported to determine MDMA and MDA content of (Concheiro et al., 2005) hair, blood, and urine (Cheze et al., 2007). LLE is time-consuming, labor-intensive, and consumes large amount of solvent, which often leads to the formation of emulsions. SPE is less time-consuming than LLE but requires column conditioning and elution with organic solvents. Bond Elut Certify columns are used for the extraction of MDMA from urine samples (Pichini et al., 2008). OASIS MCX (da Silva et al., 2010) or CBA (Meyer, Mayerhofer, Kovar, & Schmidt, 2002) columns have been used for the extraction of MDMA and its metabolites, HMA, HMMA, and MDA, in free forms from urine and plasma matrices. Other reported SPE columns which are used for extraction of MDMA and its metabolites such as MDA, HMMA, and HMA from OF, sweat, hair, plasma, and/or urine matrices are Isolute CBA column (Brunnenberg & Kovar, 2001; Buechler et al., 2003), C18AR/MPI column (Scheidweiler & Huestis, 2006), MPI column (De Martinis et al., 2007), and Clean Screen ZSDAU020 column (Martins et al., 2007).

Since LLE and SPE techniques require a significant amount of solvent, they lead to possible environmental pollution and threats to human health. In recent years, much attention has been focused on the miniaturized sample pretreatment techniques, such as solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE), and liquid-phase microextraction (LPME). The general features of all of these techniques are their easy operation and solvent-free or minimal solvent consumption. These extraction techniques are green, as well. Although SPME is simple and portable, it suffers from the comparatively expensive, fragile fiber with a limited lifetime and the sample carry-over effect. SBSE exhibits much higher sensitivity and a better detection limit than SPME, but the problems of carry-over effect and the limited lifetime of stir bar are still associated with it. Furthermore, the complex sample matrix will definitely affect the partitioning of target analytes within SPME and SBSE. LPME is an emerging technique developed from LLE, in which a small amount of solvent is employed to extract analytes from matrices. Since LPME is a simple, quick, inexpensive, and virtually solvent-free sample preparation technique, it has attracted attention and has been widely used for the analysis of organic compounds and inorganic trace elements in

environmental, biological, and food samples. The two main methodologies that evolved from the LPME approach are single drop microextraction and hollow fiber LPME (Xiong, Chen, He, & Hu, 2010). Es'haghi et al. (2010) applied triple phase suspended droplet microextraction (SD–LPME) method for cleanup and purification of hair samples containing MDMA.

Dispersive liquid–liquid microextraction is another technique that obtains appropriate detection limits (Airado-Rodriguez, Cruces-Blanco, & Garcia-Campana, 2012).

Molecular imprinted polymers (MIPs) were used in SPE as adsorbent for selective fortification of several drugs in complex matrices (Ahmadi, Ahmadi, & Rahimi-Nasrabadi, 2011). The MIPs can recognize the template molecule with affinity and selectivity comparable to those exhibited by antibodies. MIPs are inexpensive, easily prepared, and are also capable of tolerating much harsher conditions than antibodies such as, high temperatures, pressure, extreme pH, and organic solvents. Additionally, MIPs provide cleaner extractions of analytes than other usual commercial SPE columns. Ahmadi et al. prepared a MIP for extraction of MDMA from plasma samples. The monomer of this MIP was methacrylic acid and the polymerization solvent was chloroform (Ahmadi et al., 2011).

ANALYTICAL INSTRUMENTS FOR THE DETERMINATION OF MDMA AND ITS METABOLITES

As MDMA is a drug that is frequently abused, the determination of it and its metabolites is important for forensic goals, treatment of poisoning, study of its pharmacokinetics, correlating between pharmacokinetic/pharmacodynamic (PK/PD) and recognizing its side effects. Various analytical instruments and methods for the determination of MDMA and its metabolites in seized and biological samples have been developed. Although all of these instruments have advantages and drawbacks, users choose the best option for their purpose of determination. Generally, we can divide the existing analytical instruments or method for the determination of MDMA and its metabolites into qualitative and quantitative methods.

Qualitative Methods

Many various qualitative methods have been employed in the detection of MDMA and its metabolites. These methods can only be used to assess the existence of MDMA and its metabolites in the samples. Chromatography paper and color tests such as Marquis', Simon's, Chen's, and gallic acid tests are the common methods in this class.

The advantages of color tests are their simple procedures and rapid answers. However, these methods do not have high sensitivity, are not specific, and have a probability of false positive or negative results (Khajeamiri, Kobarfard, Ahmadkhaniha, & Mostashari, 2011). Mandelin reagent identifies alkaloids. MDA with this reagent changes to bluish black and in the presence of Marquis' reagent changes to blue–black (Winstock, Wolff, & Ramsey, 2001). The results of other color tests for MDA are greenish black with Froehde reagent, dark bluish green with Mecke, and light greenish yellow in the presence of nitric acid. MDMA with Simon's reagent becomes dark blue and with Marquis' reagent changes to violet–purple (Winstock et al., 2001). The gallic acid test can distinguish MDMA and MDA from amphetamine and methamphetamine due to the reaction with the methylenedioxy-substituted group. MDMA and MDA in this test produce a dark green color (Khajeamiri et al., 2011).

Paper chromatography or thin-layer chromatography (TLC) is a well-known conventional method for separation of analytes. This method is a simple, inexpensive, and applicable with many matrices. However, this method suffers from some limitations, such as low sensitivity and specificity in detection of compounds and the need for comparison with reliable standard due to low reproducibility of retention time (Kuwayama et al., 2012). To overcome these limitations, TLC is coupled with FL detection or matrix-assisted laser desorption/ionization MS (MALDI/MS).

Kato et al. reported TLC method with FL detection of MDMA and MDA in urine samples. The fluorophores developed following spraying the mixture of sodium hypochlorite, sodium hydroxide, and potassium hexacyanoferrate (III). Under UV light (250–400 nm), blue spots indicate the presence of MDMA and MDA. It is claimed that this method can quantify the amount of the analytes, as well (Kato et al., 2008).

MALDI/MS can identify large molecules such as polymers and proteins. This method needs a small volume of the sample (less than 1 μL), has sensitivity and specificity in detection of compounds, and does not need sample dilution and drug extraction. However, the limitation of this method is in the specific separation of compounds with molecular weights of lower than 500. Combinations of MALDI/MS with TLC can overcome this limitation. Kuwayama et al. developed a TLC coupled with MALDI/MS for rapid identification of MDMA, MDA, and HMMA in matrices such as rat livers, human urine, and human plasma. The obtained detection limit was 1, 0.5, and 0.005 ng/spot for MDA, HMMA, and MDMA, respectively (Kuwayama et al., 2012).

Quantitative Methods

As mentioned in section Analytical Instruments for the Determination of MDMA and Its Metabolites, determination of MDMA concentration and its metabolites in seized samples and biological fluids is important for detection of poisoning cases, determination of fatal doses, or PK/PD studies. Currently, various sensitive and specific analytical instruments are available which can be used for the determination of MDMA and its metabolites. The quantitative methods, according to their instruments, can be divided into CE, HPLC, GC, and some other novel methods. The detection techniques in these methods are MS, FL detection, UV detection, and some specific techniques for special analytical instruments.

Because MDMA is a chiral molecule (Figure 2), analytical methods that can determine the concentration of each MDMA isomer and related metabolites are preferred. The most frequently available form of MDMA is a racemic mixture. However, according to the precursor, which is used in the synthesis pathway of MDMA, the pure isomer of MDMA may be obtained.

Chiral discrimination of MDMA and its metabolites is a practical approach in either the determination of the manufacturer or PK/PD study. In the literature, researchers used chiral selector compounds, such as β-cyclodextrin (Brunnenberg & Kovar, 2001) and its derivatives such as, 2-hydroxypropyl-β-cyclodextrin (Pizarro et al., 2002) and highly sulfated γ-cyclodextrin (Iwata et al., 2013), chiral columns

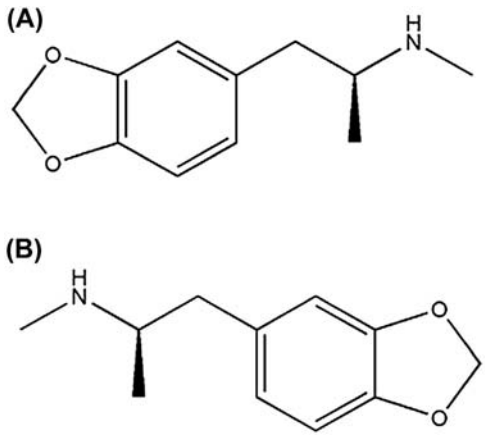

FIGURE 2 Chemical structures of MDMA enantiomers. (A) (S)-(+) 3,4-methylenedioxyamphetamine, (B) (R)-(−) 3,4-methylenedioxyamphetamine.

such as chiral-CBH (cellobiohydrolase) (Brunnenberg & Kovar, 2001; Buechler et al., 2003; Meyer et al., 2002) and derivatization with chiral agents such as (2S,4R)-*N*-heptafluorobutyryl-4-heptafluorobutoyloxy-prolyl chloride (Martins et al., 2007) for enantioselective determination of MDMA and its metabolites in plasma, hair, or other biological matrices.

Another matter in the determination of MDMA and its metabolites is the instability of MDMA metabolites with catecholic structures such as HHMA and HHA in their free forms (Jamali, Ardakani, Rouini, et al., 2013). In these cases, the determination of their free forms is almost impossible. As a result, researchers developed methods to determine the conjugated form of these metabolites or used acidic (Mueller, Peters, Huestis, Ricaurte, & Maurer, 2009) or enzymatic conjugate cleavage (Meyer et al., 2002; da Silva et al., 2010) to determine them.

High-Performance Liquid Chromatography

In the reported HPLC-based methods for the determination of MDMA and its metabolites, three kinds of detectors, MS, fluorescence, and UV, are commonly used. Electrochemical (EC) detector is reported for the determination of MDMA and its metabolite in plasma and urine matrices, as well. EC detectors can determine the concentration of MDMA and MDA as low as 1 ng/mL (Michel et al., 1993). HPLC with a UV or photodiode array detector is the routine system used in toxicological and forensic laboratories. This detector is not as sensitive and specific as MS or fluorescence detectors. To overcome these limitations, it was proposed to derivate MDMA and MDA with phenyl isothiocyanate (PITC) (Bogusz et al., 1997). The reported limit of detection for MDMA and its metabolites with this detector is between 0.1 and 3000 ng/mL (Bogusz et al., 1997; Es'haghi et al., 2010; Mc Fadden, Gillespie, Carney, & O'Driscoll, 2006). Helmlin, Bracher, Bourquin, Vonlanthen, and Brenneisen (1996) developed a method for the determination of MDMA and its metabolites in plasma and urine with UV detection, Soares et al. (2004) developed a method in urine, and Pizarro et al. (2002) developed a method in the synthesis medium. Beside the mentioned matrices, Mc Fadden et al. (2006) developed a method for the determination of MDMA and MDA in seized tablets.

As MDMA and its metabolites have native FL properties (excitation and emission wavelength is about 285 and 320 nm, respectively), detecting them with fluorescence detectors is more common among other detectors. This detector is accurate and sensitive. The sensitivity of it is more than a UV detector and almost similar to MS (Clauwaert et al., 2000; Concheiro et al., 2005; Kato et al., 2008; Meyer et al., 2002). The detection limit without derivatization for MDMA and its metabolites was about 0.8–15 ng/mL (Clauwaert et al., 2000; da Costa & da Matta Chasin, 2004; Kato et al., 2008). Some researchers derive MDMA and its metabolite by FL labeling agent such as 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoyl chloride (Meyer et al., 2002), 4-(*N*,*N*-dimethylaminosulphonyl)-7-fluoro-1,2,3-benzoxadiazole (DBD-F), dansyl chloride, or 9-fluorenylmethylchloroformate (Wada et al., 2005). Derivatization in these cases increases the sensitivity and specificity of the analytical method. Concheiro et al. (2005) developed an HPLC method for the determination of MDMA and its metabolites with fluorescence detector without derivatization in OF, Clauwaert et al. (2000) in whole blood, serum, vitreous humor, and urine, Brunnenberg and Kovar (2001) in plasma, Mancinelli, Gentili, Guiducci, and Macchia (1999) in urine, serum, saliva, and street samples, Meyer et al. (2002) in plasma, Buechler et al. (2003) in plasma and urine, da Costa and da Matta Chasin (2004) in urine, and Jamali, Ardakani, Foroumadi et al. (2013) in perfusion medium. HPLC with fluorescence detector seems to be the preferred method for the determination of MDMA and its metabolites due to its specificity, selectivity, sensitivity, and lower costs.

MS is one of the best analytical detectors, but the equipment is too expensive. HPLC with MS detector can separate the conjugated metabolites without needing hydrolysis of conjugated analytes, while this is not possible for GC–MS (Pacifici et al., 2001). In the literature, Clauwaert et al. (2000) developed a method with MS for the determination of MDMA and MDA in whole blood, serum, vitreous humor, and urine, Bogusz et al. (1997) in serum and urine, Concheiro et al. (2007) and Shima et al. (2007) in urine. Mueller, Peters, et al. (2009) describes an HPLC method with MS spectroscopy for the determination of MDMA, MDA, HHMA, and HMMA in plasma of humans and squirrel monkeys. The reported limit of detection for MDMA and its metabolites with this detector is between 0.08 and 16 ng/mL in biological fluids (Cheze et al., 2007; Mueller, Peters et al., 2009; Pichini et al., 2008) and 1–100 pg/mg in hair samples (Cheze et al., 2007; Mueller, Peters, et al., 2009). HPLC–MS as well as GC–MS is used either to validate the positive answer cases of MDMA and its metabolites (Concheiro et al., 2007) or to confirm the obtained concentrations of these compounds from other analytical methods (Clauwaert et al., 2000).

Gas Chromatography

GC is a sensitive and specific method. In case of primary and secondary amines to improve chromatographic properties, sensitivity and reproducibility, derivatization is needed. Therefore, to determine MDMA and its metabolites by GC methods derivatization is needed (Clauwaert et al., 2000; da Silva et al., 2010). Among derivatization compounds, *N*-methyl-bis(trifluoroacetamide) (Pizarro et al., 2002; Valtier, Phelix, & Cody, 2007), heptafluorobutyric acid anhydride (De Martinis et al., 2007; Scheidweiler & Huestis, 2006), or trifluoroacetic anhydride (da Silva et al., 2010)

reacts with primary and secondary amines rapidly. Also in cases of hydroxyl functional groups, *N*-methyl-*N*-trimethylsilyltrifluoroacetamide can be used (Kim et al., 2008).

Two detectors of MS spectroscopy and flame ionization detection (FID) are commonly coupled with GC for the determination of MDMA and its metabolites. GC–MS is the most widely confirmatory method used to quantify the amount of MDMA and its metabolites in positive answer cases (Concheiro et al., 2007). The reported limit of detection for MDMA and its metabolites with GC–MS is between 2 and 5 ng/mL in biological fluids (Es'haghi et al., 2010; Scheidweiler & Huestis, 2006; da Silva et al., 2010) and 30–700 pg/mg in hair samples (Es'haghi et al., 2010). Obviously, the sensitivity of FID detectors was lower than MS. The obtained detection limit for MDMA and its metabolites was 21–82 μg/mL with FID detector (Xiong et al., 2010).

Kim et al. (2008) developed a method with MS spectroscopy for determination of MDMA and MDA in fingernails, da Silva et al. (2010) for determination of MDMA, MDA, HMA, and HHMA in plasma and urine of rats, Scheidweiler and Huestis (2006) and De Martinis et al. (2007) for determination of MDMA, HMA, HMMA, and MDA in OF and sweat, respectively, and Martins et al. (2007) for determination of MDMA and MDA in hair. Xiong et al. (2010) reported a GC–FID method to determine MDMA and MDA in urine.

Capillary Electrophoresis

CE can be a considered as a replacement method for chromatography. This method needs less of the sample and solvent, and its analysis is more efficient and requires less time. Because of the small volume of the detecting chamber (about 1 μL) and the small volume of the sample, the UV detector has low sensitivity in this instrument. To overcome these shortcomings, a fluorescence detector can be used. Derivatization is probable in this method to improve the sensitivity. Zhang et al. reported a CE method with FL detection following derivatization of MDMA and MDA with fluorescein isothiocyanate. This method can detect these compounds in urine and plasma samples at concentrations as low as 0.2 ng/mL (Zhang, Wang, Yu, & Zhang, 2007). Kohler, Schappler, and Rudaz (2013) reported a CE with MS detection for determination of MDMA and MDA in urine, Iwata et al. (2013) reported a CE with UV detection of MDMA and MDA in profiling of seized samples and Airado-Rodriguez et al. (2012) for determination of MDMA in urine with UV detection. The reported detection limit with this analytical method depends on the detection technique used. The obtained detection limits for MDMA and its metabolites varied between 0.08 and 10 ng/mL (Kohler, Schappler, & Rudaz, 2013; Kohler, Schappler, Sierro, & Rudaz, 2013; Meng et al., 2011)

Miscellaneous

McAvoy, Cole, and Gueniat (1999) reported a supercritical fluid chromatographic (SFC) method with UV detection for the determination of MDMA and its metabolite, MDADue to the nonpolar nature of the SFC, the polar groups of MDMA and MDA have been derivatized with PITC or 9-fluorenylmethylcholoroformate to change MDMA and MDA to nonpolar compounds. The obtained limit of determination was about fourfold lower than GC–FID method (McAvoy et al., 1999).

A newer approach for determination of MDMA and MDA which was employed by Wada et al. is HPLC–chemiluminescence. In this method, the samples react with DBD-F in controlled conditions and an aliquot amount of the reaction mixture was injected into the HPLC system. The obtained detection limits by this method for MDMA and MDA were 3–17 and 8–58 ng/mL, respectively (Wada et al., 2012).

Near infrared spectroscopy in transmission mode is another method which is used to quantify the amount of MDMA in seized ecstasy tablets. This method is proved to be fast and precise (Schneider & Kovar, 2003). In this method, unlike the CE, liquid chromatography, and GC methods, samples are not destroyed. Because the pieces of evidence are intact, and the tablets after the determination of illegal compounds can be presented in court (Schneider & Kovar, 2003).

Since these compounds have oxidative properties, the new probable analytical method for the quantification of MDMA and MDA is a voltammetric method. Garrido et al. used a differential pulse voltammetry and squarewave voltammetry (SWV) to quantify the amount of MDMA and MDA in a seized tablet and human serum. The SWV method showed convincing correlation with the data obtained from established chromatographic methods (Garrido, Garrido, Milhazes, Borges, & Oliveira-Brett, 2010).

In almost all of the analytical methods mentioned, a sample preparation step is needed and in some cases, a derivatization step is mandatory. These limitations are not found in the technique introduced by Liu et al. They used proton nuclear magnetic resonance (NMR) with the presaturation utilizing relaxation gradient and echoes (PURGE) technique to identify and quantify the amount of MDMA in urine samples without any sample preparation and derivatization. The PURGE technique enables researchers to suppress the signal from water. The detection limit of this technique for MDMA was 10 μg/mL. The detection limit in the NMR technique depends on the number of sample scans, which, in this study was 10,000–12,000 scans. The limitation of this technique was the interference of the metabolite or other compounds in the urine when the amount of MDMA is low. In these cases, the two-dimensional sequences may be used (Liu, Decatur, Proni, & Champeil, 2010).

Recently, enzyme-linked immunosorbent assays have been introduced as a quantitative and semiquantitative method for analysis of abused drugs (Laloup et al., 2005). This method can predict the presence of MDMA and MDA in blood and OFs with more than 98% sensitivity and specificity with cutoff values of 66.5 and 51 ng/mL in plasma and OF samples, respectively (Laloup et al., 2005).

CONCLUSION

Although there are various analytical methods and instruments available for determination or identification of MDMA and its metabolites, better, faster, more specific and more sensitive analytical method is needed.

APPLICATIONS TO OTHER ADDICTIONS AND SUBSTANCE MISUSE

MDMA is commonly abused in combination with other compounds, such as psychotropic drugs, ketamine, cannabinoids, opioids, and alcohols. The determination of MDMA and its metabolites besides other illicit drugs is one the major concern of researchers. The novel analytical instruments and techniques

give a chance for simultaneous determination of these compounds. The matrices and sample preparation techniques described in this chapter can be used in the determination procedure of other illicit drugs with similar structure to MDMA.

DEFINITION OF TERMS

High-performance liquid chromatography It is an analytical method which separates compounds according to their affinity to mobile and stationary phases. In this method the mobile phase is pumped through a stationary phase (column).

Gas chromatography It is an analytical method which is used to separate compounds with the ability to vaporize without decompositions.

Capillary electrophoresis It is an analytical method which separates ionic compounds in the capillary columns via an electric field.

Ultraviolet It is an electromagnetic radiation with a wavelength of 200–400 nm.

Fluorescent It is a property of some compounds to excite by UV light and emit a visible light.

MDMA It is also known as ecstasy, an illicit psychoactive drug with an entactogenic effects.

MDA It is an *N*-demethylated metabolite of MDMA which has similar pharmacological effects to MDMA. This compound was introduced as a minor metabolite of MDMA.

HHMA It is an *O*-demethylated metabolite of MDMA which was introduced as a major metabolite of MDMA. This compound is unstable and may have a role in the neurotoxicity of MDMA.

HMA It is an MDMA metabolite which is produced from MDA and HHMA.

HMMA It is an MDMA metabolite which is produced from HHMA.

Matrix It is an analytical medium such as blood, hair, nail, etc.

Extraction It is a process to separate desirable compounds from analytical matrices.

Detection limit It is the lowest concentration which can be detected by analytical methods accurately and precisely.

KEY FACTS OF MDMA

- MDMA is an illicit psychoactive drug with an entactogenic effects.
- MDMA has two optical isomers and the pharmacokinetics of MDMA is stereoselective. Therefore, analytical methods which can detect both of these isomers and its related metabolites are preferable.
- HHMA is a major and unstable metabolite which may play a role in MDMA cell toxicity.
- HHMA can rapidly change to HMMA or conjugate with glucuronic acid.
- Rapid and accurate determination of MDMA is necessary to urgently diagnose and treat intoxication, to investigate deaths related to MDMA, to use in pharmacokinetic and pharmacodynamics studies, and to profile seized samples.

SUMMARY POINTS

- This chapter focused on the conventional and novel methods in identification, sample preparation, and determination of MDMA and its metabolites.
- The different analytical instruments such as HPLC, GC, and CE have been developed for the determination of MDMA and its metabolites.
- MDMA and its metabolites have been determined in serum, hair, nail, blood, urine, sweat, and other samples.
- The sample purification has been done via liquid–liquid extraction, SPE, and/or some newer methods, such as MIPs.
- Determination of MDMA and its metabolites needs derivatization in methods based on GC.
- According to the obtained data the HPLC with florescence detector is preferred for detection of MDMA and its metabolites due to suitable sensitivity and specificity.

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