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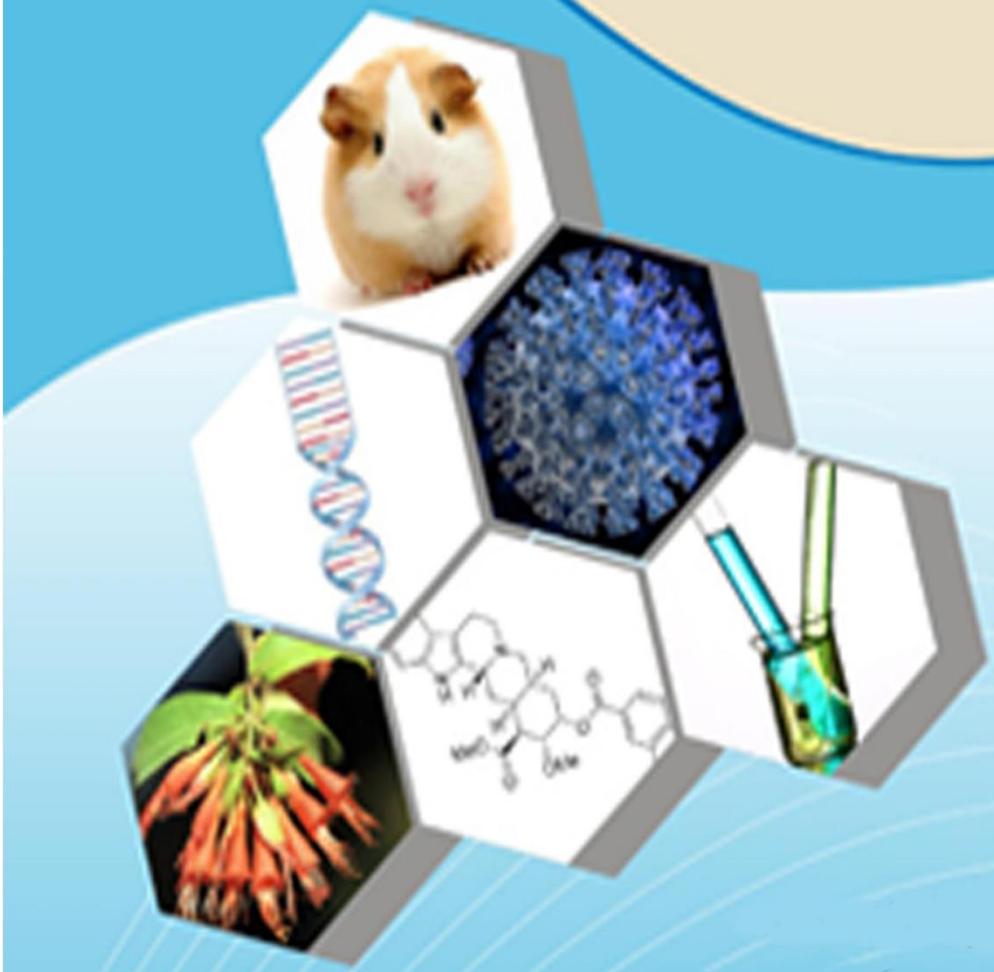

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Detection of 3-4 Methylenedioxy amphetamine from Drug Abuser's Fingers and Toenails using Liquid Chromatography with Mass Spectroscopy

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ABSTRACT

Nails have the ability to steadily collect chemicals over long periods of time, which may provide information about past drug usage and misuse. Drug analysis in human nail clippings has shown its important use in recent years for therapeutic drug monitoring, detection of drug exposure in utero, forensic toxicological applications, and program monitoring. Compared to traditional matrices (blood and urine), nails provide a number of benefits, such as an extended detection window (months to years), non-invasive sample collection, and ease of storage and transit. Because of these features, nails play a crucial role in therapeutic drug monitoring and forensic toxicology. Due to the low levels of medicines and drug addiction in nails as well as the intricate keratinized matrix, more sensitive analytical procedures are required, and sample preparation is essential. The current work aims to provide a high-performance, straightforward approach for the detection and measurement of 3,4-methylenedioxyamphetamine (MDA) in fingernail and toenail clippings using liquid chromatography-mass spectroscopy (LC-MS). Six patients receiving therapy at a rehab facility in Ujjain, Madhya Pradesh, India, had finger and toenail clippings taken. After decontaminating the nail clippings, they were hydrolyzed in 1 M NaOH at 370°C, extracted using ethyl acetate, diluted with methanol, and finally analyzed using LC-MS. Using the MDA reference standard, the calibration curve was created spanning the concentration range of 0.5 to 30 ng/mL.

Introduction

3,4-methylenedioxymetham-phetamine, or MDMA, is one of the most widely misused substances in the world. It is sometimes referred to as molly or ecstasy. This artificial material was initially created in 1912 as a raw material for hemostatic medicines. Its inception year serves as the source of its roots. Chemically speaking, the substance known as N-methyl-3,4-methylenedioxyamphetamine or 3,4-methylenedioxy-methamphetamine MDMA is known by its common or "street" name, ecstasy. [1-3] MDMA is readily absorbed from the digestive system and reaches its maximal plasma concentration around two hours after oral intake, according to Mass et al.[4] and Verebey et al. [5]. With the metabolic intermediates 3,4-dihydroxyamphetamine HHA and 3,4- hypo ethylating agents HMA, the principal metabolites of MDMA are 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methamphetamine (HMMA), and 4-hydroxy-3 methoxyamphetamine (HMA).[5–9] MDA's structure is shown in Figure 1. and the breakdown of MDMA into MDA, HMMA, and HMA, as seen in Figure 2. In recent years, there has been an increase in the number of crimes that have been made easier by the use of illegal drugs, according to the National Crime Record Bureau (NCRB, India). Numerous cases—including methamphetamine-related ones—have been reported in accordance with the NDPS Act. Occasionally, instances

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of this kind are sent to forensic science labs in order to ascertain if the corpse contained misused MDMA. [11–15] Biological matrices with shorter detection periods, such blood, urine, and saliva, are used by forensic labs to address this problem. As a result, odd, keratinized matrices—like nail samples—might be assessed for tracking the usage of illegal drugs. In addition, nail analysis may be helpful in a variety of pharmacokinetics studies, including those involving the use of drugs and alcohol during pregnancy, which can result in congenital defects, early delivery, miscarriage, higher death rates, and delayed physical and mental development.[16,17] The formation of newborn nails occurs during the second trimester of pregnancy,[18] and they continue to develop after delivery, making it possible to measure in utero drug exposure. Neonatal nails are important for identifying in-utero drug exposure to cocaine, opiates, caffeine, nicotine, and cutinize, according to research on 58 babies.[19] The ability to identify the presence of an antimycotic at the site of action during antifungal treatment for patients with onychomycosis is another use for drug analysis in nails. According to earlier studies, oral griseofulvin and ketoconazole have historically been used to treat individuals with fungal nail infections when topical treatments have failed.[20] These antifungal drugs are injected into the nail via the nail matrix, slowly integrating into the freshly produced nail, in an effort to further investigate and enhance the effectiveness of treating onychomycosis.[21, 22] Alcohol and other substance abuse have negative impacts on people as well as society. In circumstances where drug addiction is being treated, nail analysis might be useful for patient monitoring. Additionally, it may be used to officially identify individuals who relapse while receiving therapy and may need further care. One example is keeping an eye on methadone maintenance programs. Nail analysis showed promise in evaluating treatment plan compliance in patients enrolled in such programs.[23] Nevertheless, there isn't any proof as of now to back up the use of nails in treatment plans to monitor drug and/or alcohol abstinence. In fact, nail has less transportation, a broader detection window, and non-invasive sample collection.[24, 25] The current investigation employs liquid chromatography-mass spectroscopy (LC-MS) to analyze 3–4 methylenedioxyamphetamine detection from fingers and toes after three months of drug intake. As a result, the samples might be helpful for research in both forensics and medicine.

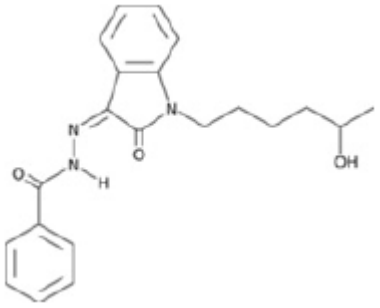

Fig. 1: 3,4-methylenedioxyamphetamine (MDA)

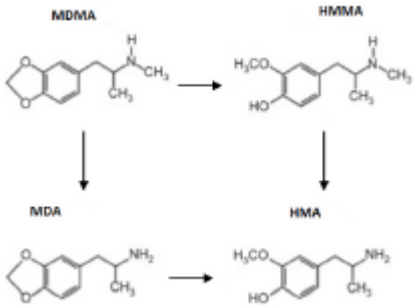

Fig. 2: Metabolism of MDMA into MDA and HMMA and HMA[8]

Material and Methods

Specimens

After ninety days of admission to Paravartan Nasha Mukti Kendra, a rehabilitation clinic in Ujjain, finger and toenail clippings were obtained from six participants. In order to eliminate uncertainty about the amount of MDA in nails caused by fluctuating nail growth rates and other reasons, samples of nails were taken from each of the five fingers and toes. Blank nail samples were graciously given by the healthy participants for recovery and spiking investigations.

Qualifications for Inclusion

Only individuals who had been admitted to the rehabilitation facility within three months of the sample collection date were taken into consideration. The only individuals taken into consideration were those who had used drugs within three months of being admitted. nFor this research, healthy individuals between the ages of 18 and 40 were taken into consideration.

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Criteria for Exclusion

The research did not take into consideration subjects who were admitted to the rehabilitation facility both before and after the date of sample collection. The research did not evaluate those who had not used drugs in the three months before to admission. Participants with any kind of physical disease or psychotic condition were not allowed to participate in this research. The research excluded those who had received any kind of cosmetic nail therapy.

Reagents and Chemicals

All of the compounds used in this study were of LC-MS quality. An analytical grade of chemicals, including sodium hydroxide, ethyl acetate, acetonitrile, and methanol, were acquired from S.K. Trader in the city of Indore, M.P., India, and Sigma Aldrich provided the standard reference solution of MDA.

The LC-MS Method

Analyte analysis was performed using the Agilent Q-TOF G6550B, which was linked to the Agilent 1260 Infinity II LC system. The Agilent Poroshell 120 HPH-C18, measuring 100 × 4.6 mm and 2.7 microns, was the dimension of the column. The temperature of the column was kept constant at 250°C. For the detection, 20 mM ammonium bicarbonate in water and methanol under gradient chromatographic conditions (Table 1) were used, as recommended by Michael SY et al.[26] The mobile phase flow rate was maintained at 0.2 milliliters per minute for a duration of 20 minutes. Electrospray ionization operating in the positive ionization mode was used for the detection. As seen in Table 2, the mass spectral parameters are tuned to get the optimum outcomes.

Sample preparation and extraction

The following stages are involved in the analysis of finger and toenail clippings: sample decontamination, nail sample digestion or extraction, and lastly, measurement of different MDA drug analyses.

Table 1: Conditions of the chromatographic gradient

Time (min)	*%Mobile phase A*	*%Mobile phase B*
0	80	20
2	80	20
9	50	50
15	0	100
16	0	100
16.5	80	20
18	80	20

Table 2: Ion source parameters

Parameter	*Range*
Nebulizer gas temperature	290°C
Sheath gas temperature	350°C
Drying gas	121/min
Nebulizer gas	35 psi
Sheath gas flow	111/min
Capillary voltage	3500 V
Nozzle voltage	1000 V

Step 1 : sample decontamination

Each finger and toenail clipping was weighed before being cleaned three times for 15 minutes at 37°C using 1 mL of dichloromethane. The layer was then disposed of, cleaned once more with water, and then treated with methanol for 15 minutes on an ultrasonicator to get rid of any contamination. Samples were then let to dry overnight in the air.

Step 2: Extracting or digesting the sample

Following an incubation period of 1 mL of 1M sodium hydroxide solution at 90°C, finger and toenail clippings were extracted using 5 mL of ethyl acetate. After drying, one milliliter of methanol was used to reconstitute the organic layer.

Step 3: Analyte quantification

Using a devised approach, an Agilent Q-TOF G6550B linked to an Agilent 1260 Infinity II LC was used to quantify a variety of analyses.

Setting up of standards

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A stock standard solution of MDA was made using methanol and kept at -180°C. The solution's concentration range was 100 ng/mL. It was discovered to be stable for six months in this situation. Six working standards were created using the stock standard solution. Nail samples that had been spiked with 200 µL of a standard MDA solution were left to dry overnight at room temperature in order to determine the best technique for sample preparation and maximal recovery. The analytical procedure was adhered to for the finger and toenail samples that were being examined, as will be discussed below. Subsequently, the residue was introduced into the LC-MS apparatus after being reconstituted to volume using 200 µL of methanol. The sanitation duration, temperature, and solvent combinations were optimized in this approach.

Method Validation

The linearity, precision, accuracy, selectivity, and sensitivity of the approach were all verified. After creating 50 mg of real spiked blank nail clippings with concentrations of0.5,0.8,1,10,12,20, and 30 ng/mL of MDA, extraction and LC-MS analysis were performed to create a standard calibration curve. The whole working range was analyzed in triplicate for linearity investigations, and the slope, intercept, and coefficient determination were determined using the least squares linear regression coefficient. The 3.3 and 10 S/N ratios, respectively, were used to derive the limits of detection and quantification.

Results

Nail samples from a single participant were extracted and subjected to the standard MDA at 20 ng/mg chromatographic conditions.

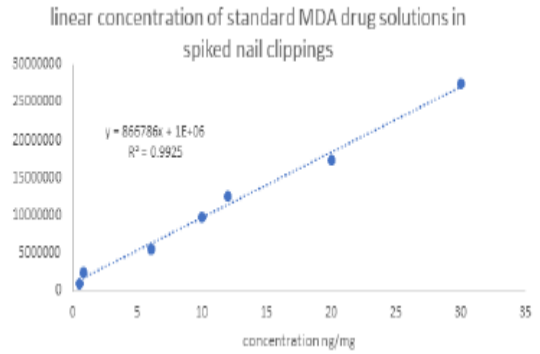

Fig. 3: Linear concentration of standard MDA drug solutions in spiked nail clippings

Table 3 summarizes data on linearity and sensitivity. For spiked nail clippings, the limits of quantification and detection were determined to be 1.21 and 4.6 ng/mg, respectively, whereas the corresponding values in the standard solution were 1.10 and 3.67 ng/mL. The measurement of MDA in nail clippings spiked at three concentration levels, such as 8, 20, and 40 ng/mg, was used to test accuracy (measured by the relative recovery percentage) (Fig. 3). Analyzing samples spiked with MDA at three concentration levels in five duplicates allowed for the evaluation of repeatability. Over the course of three days, the between-day research evaluated intermediate accuracy. With RSD levels less than 15%, the relative recovery rate fell between 95% and 105%. Data on accuracy and precision are presented in Table 4. The presence or lack of interference from the sample matrix under the same chromatographic conditions was used to measure selectivity. There was no evidence of nail matrix interference. mFigure 4 shows a typical chromatogram of MDA in extracted nail samples from drug addicts and nail clippings spiked with a concentration of 10 ng/mg.

Discussion

This study presented a fully validated method for the detection of MDA in human fingernail and toenail clippings.

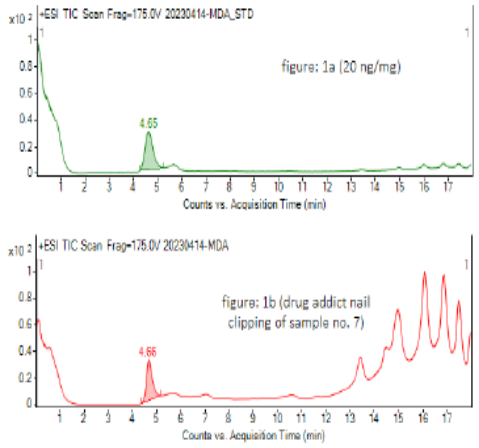

Fig. 4: Chromatograms of MDA obtained with (1a) a nail spiked with 20 ng/mg and (1b) an extracted nail sample of addict no. 7.

Table 3: Sensitivity and linearity data of the developed method

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Matrix	Linear regression	R^2	LoD	LoQ
Standard solution (ng/mL)	$y = 827470x_1$	0.993	1.10	3.67
Nail clippings (ng/mg)	$y = 866786x_2$	0.992	1.21	4.6

X_1 = Concentration of MDA in standard solution
X_2 = Concentration of MDA in spiked nail samples (in ng/mg)

Table 4: Accuracy and precision data of the developed method (within day)

Matrix	Added (ng/mg)	Within day (n=5)		
		found ± SD (ng/mg)	RSD	Recovery (%)
Nail Clippings	8	7.8 ± 0.4	5.01	98.8
	20	20.5 ± 0.4	1.9	103.0
	40	40.3 ± 0.6	1.6	101.0

Table 5: MRM transitions of MDA analyses

Analyte	(Q1) m/z	(Q2) m/z	(Q3) m/z
MDA	180	135	163.1

In this investigation, we also looked at the possibility of detecting human fingernail and toenail clippings in drug monitoring instances 90 days after drug usage. Using six samples of actual drug users, the validated technique was used to compare the detection of MDA in fingernail and toenail clippings. According to this investigation, fingernail clippings had much higher MDA concentrations than toenail clipping specimens. For fingernail and toenail clippings, the mean MDA content was 0.12 and 0.08 ng/mg, respectively. Our results for the MDA concentrations in clippings of fingernails and toenails match those of other research that have been published.[27–36]

Methamphetamine and amphetamine were found in the nails of methamphetamine users in 1984, which is the first known instance of nails being used to detect illicit drug abuse[27]. It was determined that these drugs can be detected in fingernails for up to 3 to 5 months and in toenails for up to 15 to 20 months, but the author did not provide any data pertaining to MDA drugs. The police department gave samples at random for them to use in their studies. A second investigation that found methamphetamine, amphetamine, and their metabolites in nails for up to 45 days was published three years after the first one.[28] The first study to identify amphetamine, MDA, and MDMA in a single fingernail sample was Cirimele et al.'s[29] investigation, but no time period was given. Amphetamine and methamphetamine, however, may be detected in fingernail clippings for up to 12 weeks or 3 months in 6 participants, according to David L. L. in, et al. [30]. Subsequently, Kim et al. conducted three trials in a sequence [31–33] whereby the growth rate was linked to the length of the drug detection time for both fingernail

After 90 days of drug usage, the current study's examination of six samples of MDA abusers revealed the presence of MDA with concentration ranges of 0.105 ng/mg in fingernails and 0.08 ng/mg in toenails. In this investigation, samples were not examined for MDMA molecules or other metabolites such HMMA and HMA. because MDA drug usage rather than MDMA drug use is what the drug addicts have a history of. The concentration of the substances found by the current authors and by earlier investigators is shown in Table 8. It is discovered that the established analytical approach for LC-MS-based MDA determination from nails is sensitive, quick, and accurate. From a forensic standpoint, by using the same approach in regular casework involving the same analyte, forensic toxicologists and the different law-enforcing agencies will greatly benefit from the analytical technique and assist the criminal justice system. When more traditional biological matrices, such blood or urine, are unable to demonstrate the presence of drugs, nail clippings provide an alternative biological matrix for analysis and have been shown to be effective instruments for identifying the analyze. Even after 90 days of drug use, the established approach was still able to identify the MDA medication in nail clippings.

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