Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 13, Issue 2, 2024, 100
https://doi.org/10.33263/LIANBS132.100

Implementation of Modified D-SPE Method for Simultaneous Quantification of Methamphetamine & Mephedrone Using LC-MS/MS

Anjali Jangra [1], Himanshu Khajuria [1,*], Biswa Prakash Nayak [1], Kavita Goyal [2], Rajendra Kumar Sarin [3]

[1] Amity Institute of Forensic Sciences, Amity University, Sector-125, Noida, U.P., India; anjali.jangra830@gmail.com (A.J.); hkkhajuria@amity.edu (H.K.); bpnayak@amity.edu ((B.P.N.);
[2] Forensic Science Laboratory, Rohini Sector-14, Delhi, India; kgfsldelhi@gmail.com (K.G.);
[3] National Forensic Science University, Curti, Ponda, Goa, India; sarinrk2000@yahoo.com (R.K.S.);
* Correspondence: hkkhajuria@amity.edu (H.K.);

Scopus Author ID 56639982500
Received: 26.04.2023; Accepted: 28.05.2023; Published: 30.06.2024

Abstract: New psychoactive substances (NPS) emerge on the drug market as a means to evade controlled substance legislation. Among these substances, mephedrone (MPDn) and methamphetamine (MTPn), belonging to the amphetamine category, have gained popularity as recreational drugs, especially among young people. This article presents a modified dispersive solid phase extraction (D-SPE) technique in combination with liquid chromatography and tandem mass spectrometry (LC-MS/MS) for the identification and quantification of combined with liquid chromatography and tandem mass spectrometry (LC-MS/MS) for identifying and quantifying methamphetamine and mephedrone. The method allows for the simultaneous detection of both amphetamines by integrating a clean-up procedure with LC-MS/MS technology. The method was optimized and then thoroughly validated for selectivity, linearity, precision, accuracy, and recovery capabilities. The sample solution was injected at a volume of 10 uL, and the analytical column was maintained at 40°C while variable concentrations of 5, 10, 20, 50, 100, and 200 ng/mL were used. The limit of quantification for all drugs ranged from 5.29-59.23 ng/mL, while the limit of quantification ranged from 22.27-38.31 ng/mL. Analyte recoveries ranged from 95.6% to 116%, an improvement over the previous study. This approach can be advantageous for extracting mephedrone and methamphetamine from different biological matrices as it uses a relatively small amount of solvent, ultimately decreasing the amount of waste produced.

Keywords: Mmethamphetamine; mMephedrone; quantitation; extraction; recovery; forensic toxicology.

1. Introduction

New psychoactive substances (NPS) are synthetic compounds that have psychotropic effects on the human body, similar to traditional drugs of abuse. These substances are defined according to the UN Conventions of 1961 (Single Convention on Narcotic Drugs) and 1971 (Convention on Psychotropic Substances [1]. Despite having only minor structural modifications compared to conventional drugs, new psychoactive substances (NPS) can be legally traded until national restrictions are implemented [2,3]. NPS comprise a considerable number of many stimulants that can be classified into several classified

into subgroups, including synthetic cathinones, aminoindanes, phenethylamines, piperazines, pipradrols, phencyclidine-type substances, and tryptamines. Typically, these stimulants either increase or decrease the direct or indirect elevation of neurotransmitters like noradrenaline, serotonin, and/or dopamine in the synaptic cleft [4–6].

Amphetamines, the second most commonly abused drugs, encompass a class of stimulants known for their powerful effects on the central nervous system. Acting on neurons in the brain, amphetamines, including various therapeutic agents and drugs of abuse, can create feelings of pleasure and well-being [7]. Amphetamine abuse remains prevalent worldwide, especially among adolescents and youths who require heightened energy for physically demanding tasks such as driving trucks [8]. Moreover, amphetamines are frequently used for doping during competitions. Young people are increasingly using a new type of synthetic amphetamines with modified ring systems, which are obtained from illegal sources and have become a popular drug of abuse. [9].

Mephedrone (MPDn), also known as 4-Methylmethcathinonemethylmethcathinone, is a synthetic compound marketed online as a research chemical, plant food, or "legal high" labeled "not for human consumption." It is derived from the natural cathinone (Khat). Common street names for MPDn include 4-MMC, MMCAT, and Crab. Its molecular formula is $C_{11}H_{15}NO$ and its molecular weight is 177.242 g/mol. The chemical structure of MPDn includes a phenyl ring, a methyl group, and an amino group, as shown in Figure 1. The drug produces effects similar to other psychostimulants like 3,4-methylenedioxymethamphetamine (MDMA) [10]. MPDn typically appears as a white, off-white, or yellowish powder or crystals. It has a melting point of 170-175°C and a boiling point of 259-260°C. The drug is soluble in water and organic solvents like ethanol and has a pKa value of 8.77. MPDn is a central nervous system (CNS) stimulant that produces effects similar to other psychostimulant drugs like cocaine and amphetamines. It acts as a potent serotonin, dopamine, and norepinephrine reuptake inhibitor, leading to increased levels of these neurotransmitters in the brain. This results in feelings of euphoria, increased energy, heightened alertness, and decreased appetite. MPDn has a rapid onset of action, and its effects typically last 2-4 hours. The drug also has the potential for abuse and dependence and can cause a range of adverse effects, including anxiety, agitation, hallucinations, and cardiovascular complications [11,12].

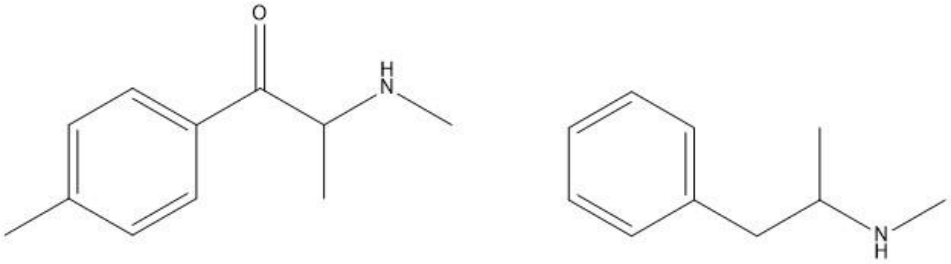

Figure 1. Chemical structures of mephedrone and methamphetamine.

Another drug in this category is mMethamphetamine (MTPn), which has the molecular formula $C_{10}H_{15}N$. Its chemical structure consists of a phenyl ring, a methyl group, and an amino group. MTPn is a highly lipophilic compound that can easily cross the blood-brain barrier, resulting in itsproducing potent CNS effects [13]. The drug acts by increasing the release of dopamine, norepinephrine, and serotonin in the brain, leading to feelings of increases the release of dopamine, norepinephrine, and serotonin in the

brain, leading to euphoria, increased energy, and decreased appetite. MTPn has a rapid onset of action, and its effects typically last several hours. The drug is highly addictive and can lead to a range of adverse effects, including anxiety, paranoia, hallucinations, and cardiovascular complications. Long-term use of MTPn can also lead to neurotoxicity and cognitive impairment [14–17].

Among patients with amphetamine consumption, suicide attempts involving a life-threatening overdose of drugs are not uncommon. Many previous works of literature report cases of toxicity and overdose of MPDn and MTPn [18–23]. Detecting illegal substances in biological samples is essential for both therapeutic and forensic purposes. However, direct detection of such substances is often difficult due to the complexity of the samples. For instance, the presence of salts, creatinine, urea, proteins, and other foreign substances in urine and saliva can impede the analysis and result in a higher matrix effect [24–28]. Artificial matrices are used to overcome such obstacles. Artificial matrices are capable of producing similar or even more accurate results than biological matrices, not only in vitro analysis [29].

Numerous techniques for analyzing amphetamines in biological samples have been documented in the literature. The methods used to analyze amphetamines in biological samples include gas chromatography-–mass spectrometry (GC-–MS) [30], liquid chromatography-–mass spectrometry (LC–-MS) [31], and capillary electrophoresis–mass spectrometry (CE-MS) [32,33]. Liquid chromatography coupled with tandem mass spectrometry (LC-–MS/MS) is still commonly used for determining multiple targets of novel psychoactive substances (NPS) [34]. When used in the multiple-reaction-monitoring mode (MRM), LC-MS/MS provides high sensitivity and selectivity, with good precision and accuracy over a wide dynamic range, enabling the development of fast analytical methods. Previously, several LC-–MS/MS applications have been described for determining a variety of NPS in biological matrices, such as serum, blood, urine, oral fluid, and hair [32–35].

For forensic and clinical purposes, this paper outlines the development and validation of developing and validating an LC-MS/MS method that utilizes D-SPE extraction to simultaneously determine two amphetamine drugs, namely MPDn and MTPn, in human urine.

2. Materials and Methods

2.1. Chemical and reagents used.

In this study, all chemicals and reagents used were of analytical grade. SIMA LABS (Sophisticated Industrial Materials Analytic Labs Pvt. Ltd.) in New Delhi, India, provided standards of MPDn and MTPn. F for HPLC (High-Performance Liquid Chromatography), methanol, water, formic acid, and ammonium formate solution were purchased from Sigma Aldrich, located in St. Louis, MO, USA. The dispersive solid-phase extraction (d-SPE) utilized an EN D-SPE salt pouch (Agilent: 5982-0650 Agilent Technologies, Inc., Santa Clara, CA, USA), along with sodium citrate tribasic dihydrate (1 g) and sodium citrate dibasic sesquihydrate (0.5 g). Anhydrous magnesium sulfate, sodium chloride, sodium acetate, and primary secondary amine (PSA) were also obtained from Sigma Aldrich, located in St. Louis, MO, USA.

2.2. Standard solutions and quality control.

Standard solutions, including stock, work, and calibration solutions, were prepared in methanol at a concentration of 0.1 mg/L and stored at a temperature of -20°C. Working saturated solutions of drugs were prepared by diluting them in methanol based on their therapeutic concentrations in artificial saliva and urine. Mix standards for calibration were prepared by serially diluting the stock solution to create working solutions at six concentration levels: 5, 10, 20, 50, 100, and 200 ng mL-1 for both drugs.

2.3. Preparation of biological matrices.

To develop an artificial eight-component urine sample, the Stolarz *et al*. (2005) protocol was followed [36]. The artificial urine contained only urea as the organic component, with a concentration of approximately 17 g/L. Additionally, it contained chlorine (9.60 g/L), sodium (5.40 g/L), sulfate (1.35 g/L), magnesium (0.65 g/L), calcium (0.20 g/L), and potassium (0.20 g/L), and was added to distilled water with a total volume of 1.0 L and a pH of 6.0. Artificial saliva was synthesized according to Pietrzyńska *et al*. (2017) according to Pietrzyńska *et al*. (2017), artificial saliva was synthesized by dissolving the entire contents in distilled water to create a total volume of 1 L [37].

2.4. D-SPE extraction protocol.

Optimization of D-SPE extraction involved implementing two strategies [38,39]. In step 1, a homogeneous artificial sample of urine and saliva spiked with MPDn and MTPn at concentrations of 5, 10, 20, 50, 100, and 200 ng/mL was mixed with 10 mL of methanol as a diluent and 10 mL of Milli Q water. The mixture was homogenized for 10 minutes on a wrist action shaker, and then an EN D-SPE salt pouch was added, and the mixture was vortexed for 1 minute before centrifugation at 6000 rpm for 10 minutes at 2-8 °C. First, 6 mL of the supernatant layer obtained from the solution was moved to a 15 mL centrifuge tube. Then, in step 2, to simplify the compound, the mixture was cleaned up using 500 mg of magnesium sulfate and 250 mg of primary and secondary amine (PSA)he mixture was cleaned up using 500 mg of magnesium sulfate and 250 mg of primary and secondary amine (PSA) to simplify the compound. The sample was vortexed for 2 minutes and centrifuged for 6 minutes at 2-8 °C and 6000 rpm. After that, the supernatant was added to a tube with 150 mg of MgSO4, vortexed, and centrifuged for 5 minutes at 12,000 rpm. Subsequently, 200 uL of the resulting extract was transferred to a different vial for further clean-up, and 10 uL of each sample was directly injected into the LCMS/MS apparatus.

2.5. Instrumentation conditions.

The LC-MS/MS analysis (Agilent 6470B, Agilent Technologies, Inc., Santa Clara, CA, USA) was conducted in positive-ion mode using the following parameters for precursor ion scans: a range of 100-500 m/z, collision energy ramping (Smart Frag) of 0.3-2.0 V, an isolation width of 4 amu for precursor ion, and analysis of one MS and one MS/MS spectrum. The analytical column, Poroshell 120 with bonded phase EC-C18 (2.7 µm, 3mm x 150 mm), was maintained at a temperature of 40°C, while the flow rate was set to 0.4 mL/min, and the sample injection volume was 10 µL. The MS/MS results were obtained using the MRM (multiple reaction monitoring) modes, which were fine-tuned for their transitions and collision energies to evaluate and quantify MPDn and MTPn.

3. Results and Discussion

3.1. Method optimization.

While the D-SPE method is descriptive and exploratory, its effectiveness relies on several factors, and the extraction method's selectivity can be modified. Hence, studies like this are crucial for evaluating particular parameters such as the sample/solvent ratio extraction solvent, pH, type and quantity of partition salts, type ofameters such as the sample/solvent ratio, extraction solvent, pH, type and quantity of partition salts, agitation, and cleaning sorbents [40,41]. This flexibility allows for changes to traditional methods and improved extraction processes [42,43].

The D-SPE method was initially evaluated in two stages, with a design experiment used to determine the optimal extraction conditions. The preliminary assessment involved comparing the amplitude and statistical significance of various parameters on the response (chromatographic peak regions) using normal plots generated for each Amphetamine. Figure 2 provides calibration plots for MPDn and MTPn in saliva and urine samples, demonstrating linear plots with a goodness of fit parameter (R^2) between 0 and 1, indicating that the regression predictions fit the data perfectly.

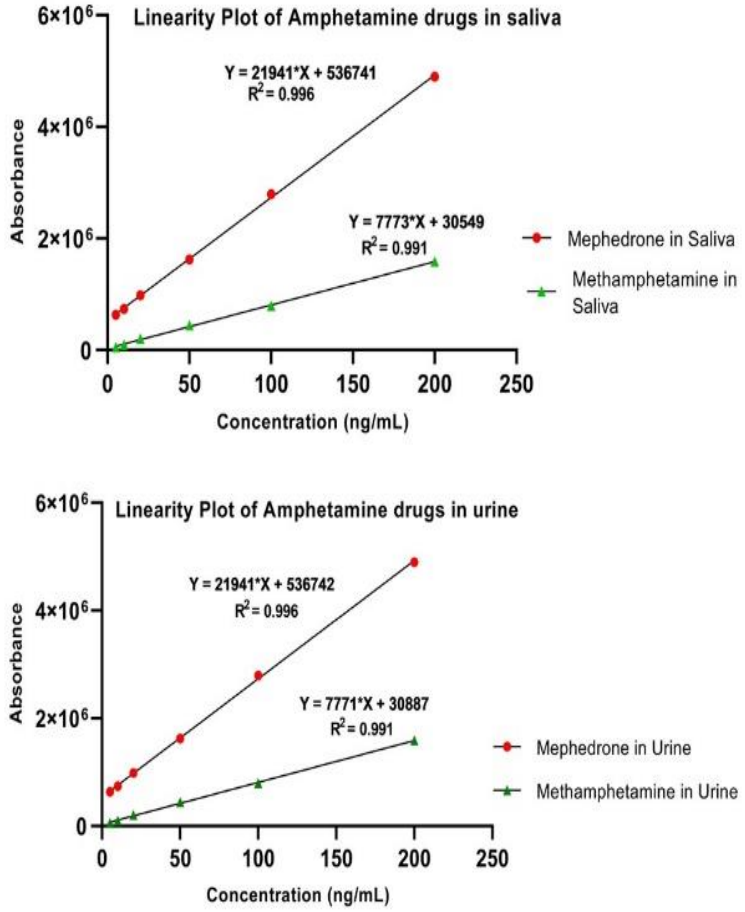

Figure 2. Linearity plots of mephedrone and methamphetamine in urine and saliva.

3.2. Implementation of LC-MS/MS conditions.

By diffusing pure standard solutions directly into the LC-MS/MS system, the optimal chromatographic and spectrometric conditions were established for MPDn and MTPn. The LC-MS/MS system detected each precursor ion before identifying two distinct product ions for each sample, employing different collision energy voltages.

The quantification specificity is significantly impacted by the composition of the mobile phase, including the concentration and pH of the buffer solution. The mobile phase's composition includes the buffer solution's concentration and pH, in addition to MS conditions. This is due to its influence on both the peak shape of the analytes in chromatography and their ionization efficiency in MS. Several studies have shown that the inclusion of 5 mM ammonium acetate in the mobile phase results in a reduction in peak width and a more symmetrical peak than water. Additionally, the signal responses of MPDn and MTPn were significantly improved by adding formic acid (0.1%).

Table 1 presents the optimal LC conditions, including retention times, for MPDn and MTPn in saliva and urine samples for MPDn and MTPn in saliva and urine samples, including retention times. The MS parameters were optimized using a 100.0 ng/mL tuning solution in positive and negative ionization modes. Results showed that both drugs exhibited substantially higher sensitivity and minimal background noise in positive ionization mode than in negative mode. The precursor/product ion mass transitions for MTPn were measured at 150.4→ 163.2 m/z, while those for MPDn were 172.1→ 178.5 m/z. Figure 3 and Figure 4 illustrate the response and retention time of both amphetamine drugs in artificial saliva and urine samples, ranging in concentration from 5 ng/mL to 200 ng/mL, as determined by LC-MS/MS.

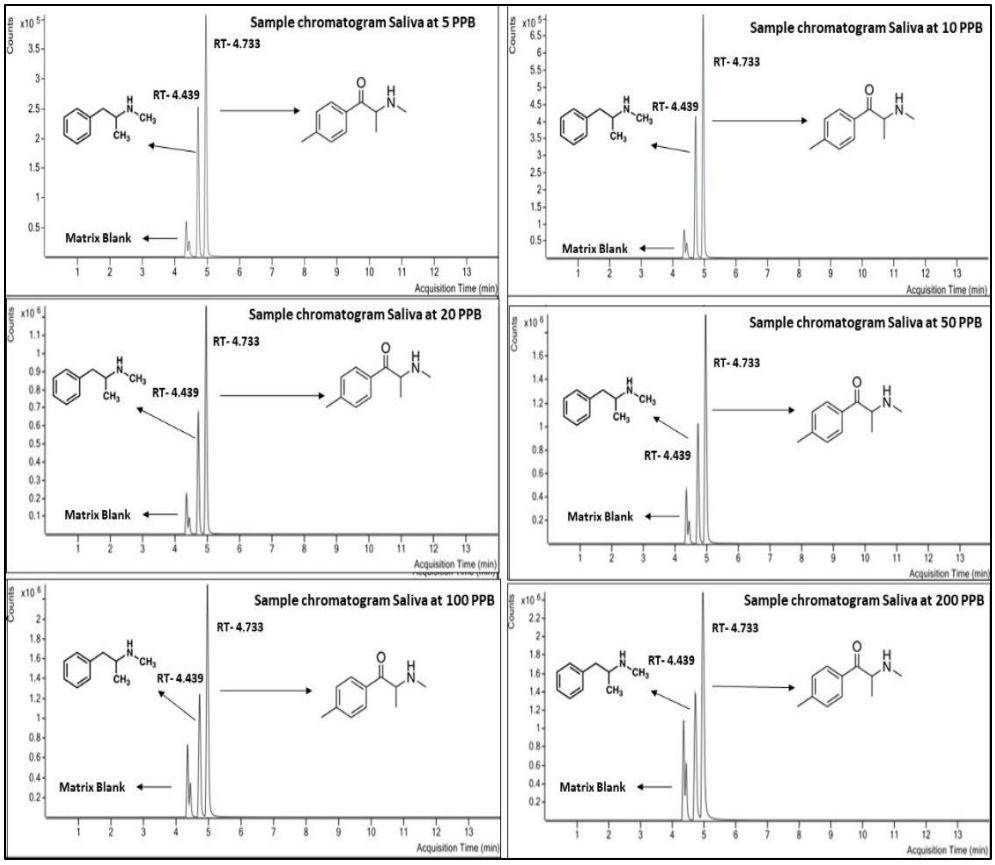

Figure 3. Obtained chromatograms for MPDn and MTPn in saliva samples at six different concentrations of 5-200 ng/ml.

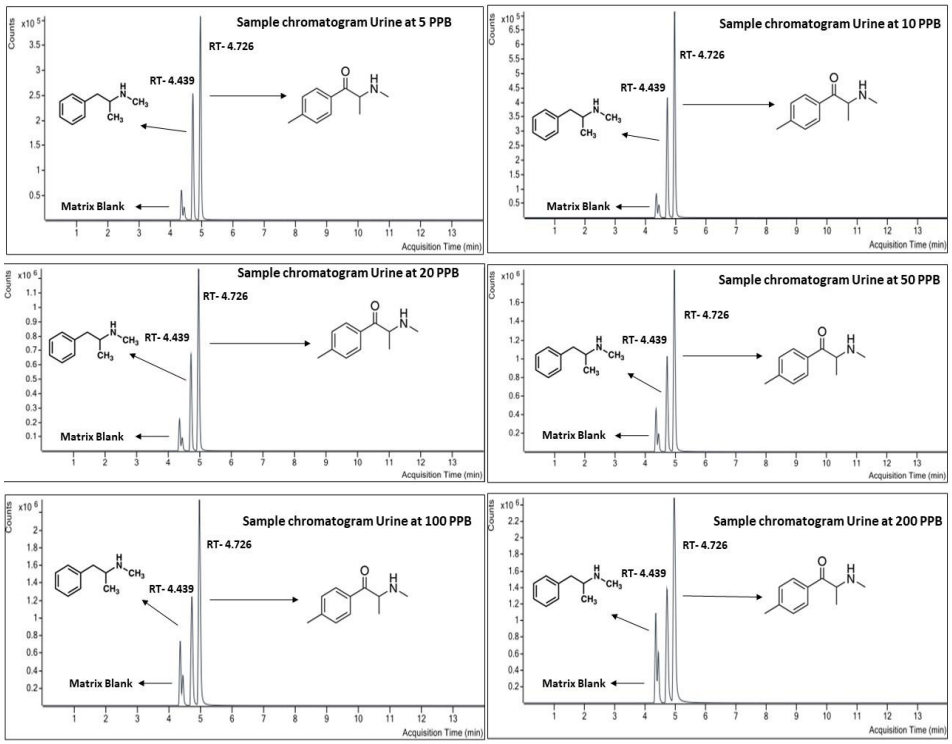

Figure 4. Obtained chromatograms for mephedrone and methamphetamine in urine samples at six different concentrations of 5-200 ng/ml.

3.3. Limit of detection and quantitation (LOD and LOQ).

To establish the linearity of the method, calibration curves were constructed using six levels of concentration and peak-area to standard internal ratios of analytes, with statistical software IBM-SPSS and GraphPad employed for analysis. The concentration range was designed to cover therapeutic concentrations for all drugs, with a working range of 10 ng/mL. Signal-to-noise ratios of 3:1 and 10:1 were used to determine LOD and LOQ, and were used to determine LOD and LOQ; the results are summarized in Table 1. Analysis revealed that the calibration range was favorable for a linear curve with 1/x2 weighting for most ADs, with a linear regression coefficient R2>0.9999, indicating a reliable level of model adjustment fit.

Table 1. LOD, LOQ, and Calibration data for mephedrone and methamphetamine in saliva and urine.

Drug	Biological matrices	R^2	Slope	Intercept	LOD (ng/mL)	LOQ (ng/mL)	RSD	Precursor (m/z)	Product (m/z)	RT
MPDn	Saliva	0.9996	21941	536741	19.79	59.93	1.31	172.1	178.5	4.726
	Urine	0.9996	9975.3	70057	5.67	17.20	1.214	172.1	178.5	4.726
MTPn	Saliva	0.9991	7773	30549	5.29	16.04	1.14	150.4	163.2	4.439
	Urine	0.9991	33357.4	704262.9	17.71	51.73	1.156	150.4	163.2	4.439

3.4 Recovery and imprecision.

Table 1 tabulates the values of slope, intercept, and standard error slope, intercept, and standard error values obtained through ANOVA analysis, which was used to scrutinize imprecision. The provided values for imprecision and recovery are consistent with those observed in prior research, and all samples evaluated met the imprecision acceptance criteria of not exceeding a variation of 20% [44,45]. The obtained recovery percentage for MPDn and MTPn ranged between 98.6 – 103.85 % for MPDn in saliva samples, 92.91 – 103.8 % for MPDn in urine samples, 85.8 – 116% for MTPn in saliva, and 95.6 – 111 % for MTPn in urine sample. All the in-between recovery ranges were tabulated in Table 2. For the smallest concentration, such as 5 ppb, the method shows great recovery for both amphetamines, which is 85.8 – 98.6 % for both matrices. However, for the injection volume of 20 ppb the method proved to be high matrix effects, which results in the method showing high matrix effects, resulting in the recovery percentage ranging from 103.8 – 116 %.

3.5 Carryover and matrix effect.

At two different concentration levels, namely the lower quality control (LQC) of 5 ng/L and higher quality control (HQC) of 200 ng/L, we recorded matrix effects for all analytes. These matrix effects exhibited less than 20%less than 20% variations for all analytes at both quality control levels, which is deemed acceptable data and complies with the validation standards. The interference test confirmed the approach's selectivity for both amphetamine drugs, as a common compound in artificial urine and saliva samples was not detected. Nonetheless, carryover was evident at the highest quantities identified from prior injections of the target drug.

Table 2. Recovery percentage of Mephedrone and Methamphetamine in saliva and urine samples

S.no	**Spiked Concentration**	**Saliva**		**Urine**	
		Extracted concentration (ng/mL)	**Recovery %**	**Extracted concentration (ng/ml)**	**Recovery %**
	Mephedrone	Extracted concentration (ng/mL)	Recovery %	Extracted concentration (ng/ml)	Recovery %
1.	5	4.93	98.6	4.93	92.91
2.	10	9.72	97.2	9.72	97.2
3.	20	20.77	103.85	20.76	103.8
4.	50	49.52	99.04	49.52	99.04
5.	100	102.43	102.43	97.17	97.17
6.	200	197.89	98.94	197.61	98.8
	Methamphetamine				
7.	5	4.29	85.8	4.29	98.24
8.	10	9.56	95.6	9.56	95.6
9.	20	23.20	116	22.20	111.0
10.	50	53.32	106.64	53.32	106.64
11.	100	97.16	97.16	102.43	102.15
12.	200	197.44	98.72	197.45	98.72

With a LOD of 5 ng/mL, the LC tandem mass spectrometer method could detect MPDn and MTPn simultaneously. Multiple protocols were employed to test biological samples, which involved involving various solvents, salt combinations, and SPE sorbents. However, limited research has optimized these conditions for the extraction of MPDn and MTPn by incorporating experiments and statistical analysis. Despite the availability of studies that involve only the first step of D-SPE extraction, which are considered method miniaturizations, we opted to test and retain the clean-up step based on the original approach. This decision was prompted by the complex matrix nature, particularly compared to other biological matrices.

Consequently, the clean-up stage was employed to eliminate any significant matrix effect, resulting in clearer extracts. In addition, efficient cleaning helps protect the HPLC column, which could potentially cause source contamination in the long term. This method also stands out as the first to exhibit excellent recovery of MPDn and MTPn in ppb levels from urine and saliva, surpassing prior LCMS/MS findings.

4. Conclusion

To summarize, the D-SPE extraction with state modification was optimized under ideal conditions, which involved using methanol as the solvent, a combination of sodium acetate and magnesium sulfate salts for salting-out effect, vortex or homogenizer for agitation, and PSA with magnesium sulfate for sorbent clean-up, with low sample and solvent consumption. The optimized approach fulfilled all the criteria for analytical attributes, indicating specificity and accuracy for MPDn and MTPn. The validation process confirmed that the approach has desirable linearity, intermediate precision, repeatability, and accuracy, with a recovery range of 95.6 to 116 percent. The method precision, in accordance with repeatability and goodness of fit parameter R2, was significant, as relative percentage standard deviation values were less than 20%. As a result, the designed framework is easy, efficient, and accurate in monitoring the lowest concentrations of MPDn and MTPn for life-threatening overdoses and suicidal behaviors. The developed method has great potential for application in clinical and forensic settings for the detection of MPDn and MTPn in biological samples. Using saliva and urine as non-invasive sample matrices also offers several advantages over other sample types, such as blood, including ease of collection, reduced risk of biohazard exposure, and reduced invasiveness.

Funding

This research received no external funding.

Acknowledgments

Declared none.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Brunt, T.M.; Nagy, C.; Bücheli, A.; Martins, D.; Ugarte, M.; Beduwe, C.; Ventura Vilamala, M. Drug Testing in Europe: Monitoring Results of the Trans European Drug Information (TEDI) Project. *Drug Test. Anal.* **2017**, *9*, 188–198, https://doi.org/10.1002/DTA.1954.
2. Stoneberg, D.M.; Shukla, R.K.; Magness, M.B. Global Methamphetamine Trends: An Evolving Problem. *Int. Crim. Justice Rev.* **2018**, *28*, 136–161, https://doi.org/10.1177/1057567717730104.
3. Schifano, F.; Orsolini, L.; Duccio Papanti, G.; Corkery, J.M. Novel Psychoactive Substances of Interest for Psychiatry. *World Psychiatry* **2015**, *14*, 15–26, https://doi.org/10.1002/WPS.20174.
4. Zhang, Y.; Zhao, Y.G.; Zhu, Y.; Shi, J.W.; Cai, T.; Zhang, S. Simultaneous Determination of 12 Illicit Drugs in Human Plasma by the PRiME Clover-Shaped Nano-Titania Functionalized Covalent Organic Frameworks Pass-through Clean-up Procedure Followed by Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry. *J. Chromatogr. A* **2022**, *1671*, https://doi.org/10.1016/j.chroma.2022.463022.
5. Song, Y.; Zhang, Y.; Wang, L.; Hu, C.; Liu, Z.F.; Feng, X.S.; He, Z.W. Cocaine in Different Matrices: Recent Updates on Pretreatment and Detection Techniques. *Crit. Rev. Anal. Chem.* **2022**, 1–20, https://doi.org/10.1080/10408347.2022.2087467.
6. Pang, B.; Zhang, Y.; Zhou, Y.; Liu, Z. fei; Liu, X. jun; Feng, X. song Recent Update on Pretreatment and Analysis Methods of Buprenorphine in Different Matrix. *Crit. Rev. Anal. Chem.* **2022**, https://doi.org/10.1080/10408347.2022.2111196.
7. McKetin, R.; Leung, J.; Stockings, E.; Huo, Y.; Foulds, J.; Lappin, J.M.; Cumming, C.; Arunogiri, S.; Young, J.T.; Sara, G.; *et al*. Mental Health Outcomes Associated with of the Use of Amphetamines: A Systematic Review and Meta-Analysis. *EClinicalMedicine* **2019**, *16*, 81–97, https://doi.org/10.1016/j.eclinm.2019.09.014.
8. Davis, J.M.; Francis Schlemmer, R. The Amphetamine Psychosis. In *Amphetamines and Related Stimulants: Chemical, Biological, Clinical, and Sociological Aspects*; CRC Press, **2019**, 161–174, https://www.taylorfrancis.com/chapters/edit/10.1201/9780429279843-11/amphetamine-psychosis-john-davis-francis-schlemmer.
9. Castaldelli-Maia, J.M.; Wang, Y.P.; Brunoni, A.R.; Faro, A.; Guimarães, R.A.; Lucchetti, G.; Martorell, M.; Moreira, R.S.; Pacheco-Barrios, K.; Rodriguez, J.A.B.; *et al*. Burden of Disease Due to Amphetamines, Cannabis, Cocaine, and Opioid Use Disorders in South America, 1990–2019: A Systematic Analysis of the Global Burden of Disease Study 2019. *The Lancet Psychiatry* **2023**, *10*, 85–97, https://doi.org/10.1016/S2215-0366(22)00339-X.
10. Dargan, P.I.; Sedefov, R.; Gallegos, A.; Wood, D.M. The Pharmacology and Toxicology of the Synthetic Cathinone Mephedrone (4-Methylmethcathinone). *Drug Test. Anal.* **2011**, *3*, 454–463, https://doi.org/10.1002/dta.312.
11. Camuto, C.; Guglielmelli, A.; De-Giorgio, F.; de la Torre, X.; Mazzarino, M.; Marti, M.; Botrè, F. In Vitro Metabolic Profile of Mexedrone, a Mephedrone Analog, Studied by High- and Low-Resolution Mass Spectrometry. *Drug Test. Anal.* **2022**, *14*, 269–276, https://doi.org/10.1002/dta.3179.
12. Pospíšilová, E.; Paškanová, N.; Kuchař, M.; Shishkanova, T. V. Potentiometric Determination of Mephedrone in Oral Fluids with Ion-Selective Membranes. *Electroanalysis* **2023**, https://doi.org/10.1002/elan.202200468.
13. Sankaran, D.; Lakshminrusimha, S.; Manja, V. Methamphetamine: Burden, Mechanism and Impact on Pregnancy, the Fetus, and Newborn. *J. Perinatol.* **2022**, *42*, 293–299, https://www.nature.com/articles/s41372-021-01271-8.
14. Tobolski, J.; Sawyer, D.B.; Song, S.J.; Afari, M.E. Cardiovascular Disease Associated with Methamphetamine Use: A Review. *Heart Fail. Rev.* **2022**, *27*, 2059–2065, https://doi.org/10.1007/s10741-022-10261-7.
15. Chen, Y.; Wisner, A.S.; Schiefer, I.T.; Williams, F.E.; Hall, F.S. Methamphetamine-Induced Lethal Toxicity in Zebrafish Larvae. *Psychopharmacology (Berl).* **2022**, *239*, 3833–3846, https://doi.org/10.1007/s00213-022-06252-z.
16. Kennis, B.; Aaser, A.; Lasoff, D.; Sweeney, D.A.; Wardi, G. Brief Report: The Diagnostic Utility of Procalcitonin Is Limited in the Setting of Methamphetamine Toxicity. *Am. J. Emerg. Med.* **2022**, *54*, 36–40, https://doi.org/10.1016/j.ajem.2022.01.049.
17. Harnett, J.T.; Dargan, P.I.; Dines, A.M.; Archer, J.R.H.; Greene, S.L.; Hunter, L.J.; Wood, D.M. Increasing Emergency Department Attendances in Central London with Methamphetamine Toxicity and Associated Harms. *Emerg. Med. J.* **2022**, *39*, 463–466, https://doi.org/10.1136/emermed-2020-209550.
18. Darke, S.; Kaye, S.; Duflou, J.; Lappin, J. Completed Suicide Among Methamphetamine Users: A National Study. *Suicide Life-Threatening Behav.* **2019**, *49*, 328–337, https://doi.org/10.1111/sltb.12442.
19. Dickson, A.J.; Vorce, S.P.; Levine, B.; Past, M.R. Multiple-Drug Toxicity Caused by the Coadministration of 4-Methylmethcathinone (Mephedrone) and Heroin. *J. Anal. Toxicol.* **2010**, *34*, 162–168, https://doi.org/10.1093/jat/34.3.162.
20. Troya, J.; Martínez de Gándara, A.; Ryan, P.; Cuevas, G.; Pardo, V. Mephedrone and Chemsex: When It Stops Being a Party and Becomes a Fatal Problem. *Int. J. STD AIDS* **2019**, *30*, 1028–1030, https://doi.org/10.1177/0956462419857004.
21. Darke, S.; Kaye, S.; Duflou, J.; Lappin, J. Completed Suicide Among Methamphetamine Users: A National Study. *Suicide Life-Threatening Behav.* **2019**, *49*, 328–337, https://doi.org/10.1111/sltb.12442.

22. Debruyne, D.; Loilier, M.; Cesbron, A.; Le Boisselier, R.; Bourgine, J. Emerging Drugs of Abuse: Current Perspectives on Substituted Cathinones. *Subst. Abuse Rehabil.* **2014**, *37*, https://doi.org/10.2147/sar.s37257.
23. Troya, J.; Martínez de Gándara, A.; Ryan, P.; Cuevas, G.; Pardo, V. Mephedrone and Chemsex: When It Stops Being a Party and Becomes a Fatal Problem. *Int. J. STD AIDS* **2019**, *30*, 1028–1030, https://doi.org/10.1177/0956462419857004.
24. Lukić, V.; Micić, R.; Arsić, B.; Nedović, B.; Radosavljević, Ž. Overview of the Major Classes of New Psychoactive Substances, Psychoactive Effects, Analytical Determination and Conformational Analysis of Selected Illegal Drugs. *Open Chem.* **2021**, *19*, 60–106, https://www.degruyter.com/document/doi/10.1515/chem-2021-0196/html?lang=en.
25. Elboraie, M.Z.; Elbashir, A.A.; Eid, E.E.M.; Aboul-Enein, H.Y. Dispersive Solid-Phase Extraction for Simultaneous Determination of Four Amphetamines Drugs in Urine Using Gas Chromatography–Mass Spectrometry. *J. Iran. Chem. Soc.* **2022**, *19*, 753–762, https://doi.org/10.1007/s13738-021-02339-0.
26. Krotulski, A.J. Target Analysis of Synthetic Cannabinoids in Blood and Urine. In *Methods in Pharmacology and Toxicology*; **2023**; pp. 1–19, https://link.springer.com/protocol/10.1007/978-1-0716-2605-4_1.
27. Roszkowska, A.; Plenis, A.; Kowalski, P.; Bączek, T.; Olędzka, I. Recent Advancements in Techniques for Analyzing Modern, Atypical Antidepressants in Complex Biological Matrices and Their Application in Biomedical Studies. *TrAC - Trends Anal. Chem.* **2022**, *152*, 116609, https://doi.org/10.1016/j.trac.2022.116609.
28. Elboraie, M.Z.; Elbashir, A.A.; Eid, E.E.M.; Aboul-Enein, H.Y. Dispersive Solid-Phase Extraction for Simultaneous Determination of Four Amphetamines Drugs in Urine Using Gas Chromatography–Mass Spectrometry. *J. Iran. Chem. Soc.* **2022**, *19*, 753–762, https://doi.org/10.1007/s13738-021-02339-0.
29. Singh, C.; Sharma, J.; Nayak, B.P.; Pandya, P.; Singh, G.K.; Khajuria, H. In-Vitro Quantification of Tramadol and Tianeptine Antidepressants from Biological Matrices Using Modified QuEChERS and LC-MS/MS. *Mater. Today Proc.* **2023**, https://doi.org/10.1016/j.matpr.2023.02.134.
30. Jadhav, M.R.; Pudale, A.; Raut, P.; Utture, S.; Ahammed Shabeer, T.P.; Banerjee, K. A Unified Approach for High-Throughput Quantitative Analysis of the Residues of Multi-Class Veterinary Drugs and Pesticides in Bovine Milk Using LC-MS/MS and GC–MS/MS. *Food Chem.* **2019**, *272*, 292–305, https://doi.org/10.1016/j.foodchem.2018.08.033.
31. Kahl, J.M.M.; da Cunha, K.F.; Rodrigues, L.C.; Chinaglia, K. de O.; Oliveira, K.D.; Costa, J.L. Quantification of Amphetamine and Derivatives in Oral Fluid by Dispersive Liquid-Liquid Microextraction and Liquid Chromatography—Tandem Mass Spectrometry. *J. Pharm. Biomed. Anal.* **2021**, *196*, https://doi.org/10.1016/j.jpba.2021.113928.
32. De Jager, A.D.; Bailey, N.L. Online Extraction LC-MS/MS Method for the Simultaneous Quantitative Confirmation of Urine Drugs of Abuse and Metabolites: Amphetamines, Opiates, Cocaine, Cannabis, Benzodiazepines and Methadone. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2011**, *879*, 2642–2652, https://doi.org/10.1016/j.jchromb.2011.07.029.
33. Dai, Z.; Valdez, L.; Dumit, M. Chiral Analysis of Methamphetamine and Related Controlled Substances in Forensic Science. In *books.google.com*; 2019; pp. 1–39.
34. Accioni, F.; García-Gómez, D.; Girela, E.; Rubio, S. SUPRAS Extraction Approach for Matrix-Independent Determination of Amphetamine-Type Stimulants by LC-MS/MS. *Talanta* **2018**, *182*, 574–582, https://doi.org/10.1016/j.talanta.2018.02.039.
35. Khajuria, H.; Nayak, B.; Medicine, A.B.-; The, S. and; 2018, U. Toxicological Hair Analysis: Pre-Analytical, Analytical and Interpretive Aspects. *journals.sagepub.com*. , https://doi.org/10.1177/0025802418768305.
36. Stolarz, A.; Alonso, A.; Bolle, W. De; Kühn, H.; Richter, S.; Quetel, C.; Ponzevera, E.; Verbruggen, A.; Wellum, R. Preparation of Simulated Urine Samples Containing Certified Uranium for the NUSIMEP 4 Campaign. *Ec.Europa.Eu* **2015**. https://joint-research-centre.ec.europa.eu/system/files/2013-01/nusimep4_reportonpreparationofsamples.pdf.
37. Pietrzyńska, M.; Voelkel, A. Stability of Simulated Body Fluids Such as Blood Plasma, Artificial Urine and Artificial Saliva. *Microchem. J.* **2017**, *134*, 197–201, https://doi.org/10.1016/j.microc.2017.06.004.
38. Mohebbi, A.; Yaripour, S.; Farajzadeh, M.A.; ... Combination of Dispersive Solid Phase Extraction and Deep Eutectic Solvent–Based Air–Assisted Liquid–Liquid Microextraction Followed by Gas Chromatography–Mass *Journal Of Cromatography A* **2018**, *1571*, 84-93, https://doi.org/10.1016/j.chroma.2018.08.022.
39. Islas, G.; Ibarra, I.S.; Hernandez, P.; Miranda, J.M.; Cepeda, A. Dispersive Solid Phase Extraction for the Analysis of Veterinary Drugs Applied to Food Samples: A Review. *Int. J. Anal. Chem.* **2017**, *2017*, 753–762, https://www.hindawi.com/journals/ijac/2017/8215271/.
40. Bahrani, S.; Ghaedi, M.; Asfaram, A.; Mansoorkhani, M.J.K.; Javadian, H. Rapid Ultrasound-Assisted Microextraction of Atorvastatin in the Sample of Blood Plasma by Nickel Metal Organic Modified with Alumina Nanoparticles. *J. Sep. Sci.* **2020**, *43*, 4469–4479, https://doi.org/10.1002/JSSC.202000660.
41. Kumar Singh, G.; Kaur, R.; Singh, C.; Singh, P. Elemental Analysis and Characterization of Vermilion and Lipstick Sample Using SEM-EDS. *Mater. Today Proc.* **2022**, https://doi.org/10.1016/j.matpr.2022.12.180.
42. Rani, M.S.S.; Singh, C.; Mishra, A. Development of an Analytical Method for Detection of Imidacloprid Insecticide from Biological Matrix Using LC-MS/MS. *J. Punjab Acad. Forensic Med. Toxicol.* **2021**, *21*, 220–225, https://doi.org/10.5958/0974-083x.2021.00042.x.
43. Singh, C.; Rani, M.S.S.; Mishra, A. Development of Analytical Method for Detection of Monocrotophos Insecticide from Biological Matrix Using Lc-Ms/Ms. *Int. J. Med. Toxicol. Leg. Med.* **2020**, *23*, 210–219, https://doi.org/10.5958/0974-

4614.2020.00031.5.
44. Labat, L.; Deveaux, M.; Dallet, P.; Dubost, J.P. Separation of New Antidepressants and Their Metabolites by Micellar Electrokinetic Capillary Chromatography. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2002**, *773*, 17–23, https://doi.org/10.1016/S1570-0232(01)00623-7.
45. Montenarh, D.; Wernet, M.P.; Hopf, M.; Maurer, H.H.; Schmidt, P.H.; Ewald, A.H. Quantification of 33 Antidepressants by LC-MS/MS - Comparative Validation in Whole Blood, Plasma, and Serum. *Anal. Bioanal. Chem.* **2014**, *406*, 5939–5953, https://doi.org/10.1007/s00216-014-8019-x.