MedCrave
Step into the World of Research
Forensic Research & Criminology International Journal

Review Article
Open Access
CrossMark

Microextraction techniques for forensic drug analysis in saliva

Abstract

Forensic drug analysis involves trace level analysis of drugs of abuse and their metabolites in different biological matrices. Saliva has proven to be an alternative matrix over blood, plasma and urine, since collection of saliva is non-invasive, simple, and rapid. Additionally, concentration of drugs in saliva can be correlated to free drug concentration in plasma. Recently, great attention has been paid to develop microextraction techniques for analysis of drugs of abuse in saliva in order to improve quality and sensitivity of analysis. Microextraction techniques are rapid, eco-friendly, inexpensive, simple and offer high extraction efficiencies and enrichment factors. This article focuses on the reviews of procedures and applications of modern microextraction techniques for analysis of drugs of abuse in saliva samples.

Keywords: forensic drug analysis, microextraction technique, drugs of abuse, saliva, solid-phase microextraction, dispersive liquid-liquid microextraction, microextraction with packed sorbents

Volume 5 Issue 4 - 2017

Rajeev Jain
Forensic Toxicology Division, Central Forensic Science Laboratory, India

Correspondence: Rajeev Jain, Forensic Toxicology Division, Central Forensic Science Laboratory, 16, LachitBorphukan Path, Tetelia, Gotanagar, Guwahati -781033, Assam, India, Tel 91-361-2571149, Fax 91-361-2571148,
Email rajeevjainccfsl@gmail.com , Rajeev.jain-as@gov.in

Received: August 01, 2017 | **Published:** October 13, 2017

Introduction

Forensic drug analysis involves qualitative and quantitative analysis of drugs of abuse and their metabolites in various complex biological matrices such as biological fluids and seized materials. Drugs of abuse such as opioids, cannabinoids, cocaine, amphetamines, hallucinogens and benzodiazepines are generally analyzed in biological fluids e.g. urine and plasma. While urine is efficient for investigation of metabolites, plasma reflects total concentration of drug (bound and unbound).[1]In recent times, saliva has emerged as an alternative biological matrix for screening and determination of drugs of abuse. Main advantages of saliva drug testing are,

a. Sampling of saliva is simple, quick and non-invasive and can be performed on-site,

b. In case of sampling supervision, unlike sampling of urine, saliva sampling can be done without violating any privacy, and

c. Concentration of drugs in saliva can be correlated to free-drug concentration in plasma.[2,3]

Microextraction techniques (MTs) are basically miniaturized forms of extraction techniques where zero or microliters of extraction solvent and small amount of sample is used for extraction of target analytes. MTs offer manifold advantages over widely used conventional liquid-liquid extraction (LLE) and solid-phase extraction (SPE) viz.[4]

i. Consumes microliters or zero volume of toxic organic solvents, e.g. liquid-phase microextraction (LPME) and solid-phase microextraction (SPME),

ii. Easy to perform and offers high enrichment factors and extraction efficiencies,

iii. In case of polar analytes simultaneous derivatization and preconcentration is possible,

iv. Unlike LLE and SPE, there is no need of additional evaporation and preconcentration step,

v. Extract is clean with least matrix interference, and

vi. Compatible with different analytical instruments.[5]

Broadly, MTs can be classified into two categories i.e. sorbent based MTs and solvent based MTs. In sorbent based MTs, analytes are allowed to be adsorbed on a solid/sorbent stationary phase e.g. SPME, microextraction with packed sorbent (MEPS), polymer monolithic microextraction (PMME), whereas in solvent based MTs, microliters of extraction solvent is used to extract the analyte from matrix, e.g. DLLME.[6] Since the introduction of MTs, they have been widely applied for analysis of drugs of abuse from various matrices.[7,8]The present review focuses on applications of MTs for analysis of drugs of abuse particularly from saliva samples. Table 1 shows a brief summary of applications of MTs for forensic drug analysis.

Table 1 Application of various MTs for forensic drug analysis

Drugs of abuse	Microextraction Technique	Detection technique	LOD	Ref
AMP & MAMP	DI-SPME	GC-MS	5 and 0.5ng/mL	10
MDA, MDMA, MDEA & MBDB	HS-SPME	GC-MS	3.14, 1.19, 1.17 & 1.92ng/mg	11
AMP, MAMP, MDA and MDMA	SVLE	GC-MS	1, 5, 2 & 3ng/mL	12
AMP, MAMP, MDA, MDMA and MDEA	MEPS	LC-MS/MS	1, 1, 1, 0.5 & 0.5ng/mL	13
Δ9-THC, Δ8-THC, CBD and CBN	DI-SPME	IT-GC-MS	1ng/mL	14

Table Continued...

Drugs of abuse	Microextraction Technique	Detection technique	LOD	Ref
THC, CBD, CBN	SPME	GC-MS	-	15
Δ9-THC	PMME	GC-MS	0.68ng/mL	16
THC	DI-SPME	GC-MS	3ng/mL	10
Cocaine, Cocaethylene, EME, AEME	HFMSME	GC	11, 12, 28 & 6ng/mL	17
Cocaine and Cocaethylene	DI-SPME	GC-MS	20 and 5ng/mL	18
Cocaine	HS-SPME	GC-MS	5ng/mL	10
Cocaine, BZE, NCOC and EME	MEPS	LC-MS/MS	0.3, 0.8, 0.5 & 1ng/mL	13
Methadone & EDDP	DI-SPME	GC-MS	0.004 & 0.008µg/mL	19
Methadone	DLLME	HPLC-UV	25.12 ng/mL	20
Methadone, EDDP, BUP and NBUP	MEPS	LC-MS/MS	0.2, 0.3, 2 & 2ng/mL	13
Morphine, Codeine	MEPS	LC-MS/MS	2, 2 and 0.8ng/mL	13
6-MAM				
PCP, MES & psilocybin	µ-SPE	LC-MS/MS	0.1, 0.07 & 1.2ng/mL	21
MES & PCP	MEPS	LC-MS/MS	2 & 0.2ng/mL	13

AMP, amphetamine; MAMP, methamphetamine; MDA, methylenedioxyamphetamine; MDMA, methylenedioxymethamphetamine; MDEA, methylenedioxymethamphetamine; MBDB, N-methyl-1- (1,3-benzodioxol-5-yl)-2-butanamine; DI-SPME, direct immersion solid-phase microextraction; GC-MS, gas chromatography-mass spectrometry; HS-SPME, head space solid-phase microextraction; SVLE, small volume microextraction; MEPS, microextraction by packed sorbent; THC, tetrahydrocannabinol; CBD, cannabidiol; CBN, cannabinol; LC-MS/MS, liquid chromatography-tandem mass spectrometry; IT-GC-MS, ion trap GC-MS; EME, ecgonine methyl ester; AEME, anhydroecgonine methyl ester; HFMSME, hollo fiber membrane solvent microextraction; BZE, benzylecgonine; NCOC, norcocaine; EDDP, 2-ethylidine-3, 3-diphenylpyrrolidine; HPLC-UV, high performance liquid chromatography ultraviolet detection; BUP, buprenorphine; NBUP, norbuprenorphine; 6-MAM, 6-monoacetylmorphine; PCP, phencyclidine; MES, mescaline; µ-SPE, micro solid-phase extraction

Applications of MTs for analysis of drugs of abuse in saliva samples

Analysis of amphetamines

Amphetamines (AMP) are strong central nervous system stimulants. Amphetamine and methamphetamine (MAMP) are two prominent drugs of this class. Various substitutions in the structure of these two drugs have been made in order to limit their side effects and to maintain anorexic activity.[9] SPME-gas chromatography-mass spectrometry (SPME-GC-MS) method has been developed for analysis of AMP and MAMP along with other drugs of abuse in saliva samples. Since, AMP and MAMP are polar drugs; they need to be derivatized prior to GC-MS analysis. Butylchloroforamte was used as derivatizing reagent. Non-polar derivatives thus formed were subjected to SPME using polydimethylsiloxane (PDMS) fiber for 20min under magnetic stirring. In this way, analytes were adsorbed on PDMS sorbent. These analytes were then desorbed in heated injection port of GC-MS for 15min. The method was found to be sensitive enough and offered limit of detections of 5 and 0.5ng/mL for AMP and MAMP, respectively.[10]

Gentili and other researchers reported a head-space SPME-GC-MS method for simultaneous analysis of AM like drugs in hair and saliva samples. For saliva sample, no sample pre-treatment was needed. Saliva samples were heated in SPME vial for 20 minutes at 70°C (pre-incubation period) which allowed analytes to come into vapor phase in the head-space of SPME vial. This was followed by adsorption of analytes on SPME fiber (100µm PDMS) for five minutes at 70°C. In this method, since analytes were pre-heated before SPME procedure, derivatization of AMPs was not required. The LODs for methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methylenedioxyethamphetamine (MDEA) and N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB) were found to be in range of 1.17 - 3.14ng/mg.[11]

Small volume liquid extraction (SVLE) method was developed for AMP, MAMP, MDA and MDMA in saliva samples. The proposed method utilized, only 100µL of extraction solvent (either cyclohexane or chloroform) for extraction of analytes from 1mL of saliva samples. The procedure is simple and includes ultrasonication for five minutes followed by centrifugation. OneµL of organic phase was either directly injected into GC-MS or subjected to derivatization with N-Methyl-bis-trifluoroacetamide (MBTFA) and then analyzed. The method was compared with conventional liquid-phase extraction and offered high enrichment factors. Additionally, the procedure does not involved steps like sample transfer and evaporation, which resulted in increased sensitivity. The LODs for tested analytes were found to be less than 5ng/Ml.[12] Recently, another MT i.e. MEPS for determination of AMPs along with other drugs of abuse has been proposed. MEPS is basically miniaturized form of SPE where the sorbent bed is reduced to few milligrams and inserted into a syringe barrel. Before passing saliva samples through MEPS syringe, preconditioning of the same was achieved by passing methanol and methanol/water (80:20v/v). This is followed by passing saliva sample supernatant five times (dispensing and aspirating the same aliquot) through MEPS syringe. In this way, retained analytes were eluted using 5mM formic acid in methanol after washing the MEPS syringe with 50 mMNH_3 in water/methanol.[13]

Analysis of cannabinoids

Cannabinoids are psychoactive constituents of flowering plant cannabis. Principal cannabinoids are Δ9-tetrahydrocannabinol (Δ9-THC), cannabinol (CBN) and cannabidiol (CBD), although, CBD is not psychoactive. MTs for analysis of cannabinoids have been

recently reviewed.[6] Hall and other researchers,[14]for the first time reported a DI-SPME method for extraction of Δ9-THC, Δ8-THC, CBD and CBN from saliva samples. Saliva samples were diluted and treated with acetic acid in order to deproteinize them. The PDMS fiber of 100 µm thickness was immersed into saliva sample for 10 min. After extraction, the fiber was inserted in Ion Trap-GC-MS injection port for 12min at 270°C. Addition of acetic acid in saliva samples enhanced recovery of cannabinoids by four to seven times. In another study, saliva samples were collected using EPITOPE device obtained from EPITOPE Inc. 200µL of saliva was obtained after centrifugation of EPITOPE and directly subjected to SPME using 30µm PDMS coating without any sample pretreatment. Analytes were thermally desorbed in GC-MS injection port at 240°C for 1 min.[15]

Luo and other researchers,[16]described a polymer monolithic microextraction (PMME) method for determination of Δ9-THC in saliva samples. In PMME, extraction is performed inside a monolithic capillary column coated with poly(methacrylic acid-co-ethylene glycol dimethacrylate) (p(MAA-co-EGDMA)). Extraction procedure involves four steps comprising of preconditioning, sorption of analyte on capillary column, clean-up and desorption of analyte. Monolithic structure inside the capillary column offers larger surface area in comparison to SPME, thus achieving high extraction efficiencies. The LOD for Δ9-THC was found to be 0.68ng/mL. The method was found to be rapid and consumed less than 20min. Yonamineet al.[10], extracted THC from saliva samples by submersing 100 µm PDMS fiber directly into saliva samples acidified with glacial acetic acid for 20min under magnetic stirring. After extraction, SPME fiber was injected into GC-MS injection port for 15min in order to complete desorption of THC.

Analysis of cocaine

Cocaine, an odorless white crystalline powder obtained from the leaves of Erythroxylum Coca, is a strong central nervous system stimulant. A hollow fiber membrane solvent microextraction (HFMSME) method was developed for screening of cocaine and its metabolites viz. cocaine, cocaethylene, ecgonine methyl ester (EME) and anhydroecgonine methyl ester (AEME), from saliva samples. A polypropylene hollow fiber was preconditioned and filled with extraction solvent (chloroform, 10µL). One end of the fiber was sealed. Following pH adjustment of the sample to 10.5, extraction was performed under constant stirring for 10 min. Four µL of the extraction solvent was taken back into syringe and injected into GC system for analysis. Detection limits were found to be in the range of 6-28ng/mL.[17] Both DI-SPME and HS-SPME have been compared for the quantitative determination of cocaine and cocaethylene in saliva samples. In case of DI-SPME, 30µm PDMS fiber was directly immersed into saliva sample and extraction was performed at room temperature for 30 minutes.

On the other hand, HS-SPME was performed for 45 minutes at 60°C. Desorption of analytes were taken at 250°C for one minute. Results shown that, DI-SPME offered lower detection limits for cocaine and cocaethylene in contrast to HS-SPME.[18] HS-SPME procedure described in reference[10] has also been applied for determination of cocaine in saliva. The sampling procedure for cocaine was similar to AMSs except derivatization. LOD achieved for cocaine was 5ng/mL with good recovery up to 93.5 percent. Previously described MEPS method,[13] was also applied for cocaine, benzylecgonine (BZE), norcocaine (NCOC) and EME. All analytes were extracted in a single step of MEPS as described above and analyzed by LC-MS/MS.

Analysis of opioids

Opioids act on opioid receptors and produce morphine like effects. Methadone is one of the most important drugs of this category. A comparative study of LLE and SPME was reported for the determination of methadone and 2-ethylidine-3,3-diphenylpyrrolidine (EDDP) in human saliva samples. A small volume (0.1mL) of saliva sample was treated with buffer and sodium chloride followed by DI-SPME with 100µm PDMS fiber for 30 minutes. Desorption ofanalytes were performed at 250°C for one minute. This method was compared with LLE using n-hexane. Under optimized conditions, results showed that LOD offered by SPME technique for methadone and EDDP were much lower in comparison to LLE.[19] The DLLME method for determination of trace amount of methadone in saliva and other matrices (urine, plasma and sweat) was reported. In this method, a suitable mixture of extraction and disperser solvent (250µL of chloroform and 2.5mL of methanol) was rapidly injected into 10 mL of aqueous solution containing analyte (or diluted saliva sample) which resulted in the formation of a cloudy solution. Upon centrifugation of this solution, sedimented phase of chloroform was taken out, evaporated and reconstituted in methanol which was injected into HPLC for analysis. The method was rapid as it consumed less than five minutes for extraction of methadone.[20] Microextraction with packed sorbent was also reported for methadone, EDDP, buprenorphine (BUP) and norbuprenorphine (NBUP) in addition to other drugs of abuse, as described in ref.[13]Additionally, this method was also extended for determination of opiates like morphine, codeine, diacetylmorphine (DAM), 6-monoacetylmorphine (6-MAM) in saliva samples.

Analysis of hallucinogens

Hallucinogens are defined as drugs which alter the mood and perception of an individual without effecting on brain activities.[4] Phencyclidine (PCP), mescaline (MES), psilocybin and lysergic acid diethylamide (LSD) are main hallucinogen drugs. Only a few articles are available in the literature which described application of any MT for analysis of hallucinogens in saliva samples. Sergi and other researchers,[21] reported micro-solid phase extraction (µ-SPE) for extraction of hallucinogens (PCP, MES & psilocybin) along with other illicit drugs (amphetamines, cocaine, ketamine etc.) from saliva samples. In this method, µ-SPE was performed with OMIX C18 tips which were functionalized with apolar chain of octadecylsilane into monolithic structure. Extraction procedure comprises of initial conditioning of OMIX C18 tips with ultrapure water/acetonitrile and ultrapure water/methanol solution followed by passing the saliva supernatant by loading and releasing cycle with the pipette. In this way, adsorbed analytes were eluted with methanol after a washing step with ultrapure water. The eluent was analyzed by LC-MS/MS.[21]The MES and PCP were also determined by MEPS technique coupled to LC-MS/MS analysis.[13]

Conclusion and future trends

Sample preparation in analytical toxicology is one of the most time consuming, tedious and error prone tasks. Traditional sample preparation techniques such as LLE and SPE are now being replaced with modern MTs, owing to their simplicity, environment friendliness, rapidity, cost-effectiveness, extraction efficiencies, and sensitivity. Saliva has emerged as an alternative specimen for forensic drug analysis. Various MTs such as SPME, MEPS, HFMSME, DLLME

and µ-SPE have been applied for simultaneous determination of drugs of abuse in saliva samples. These MTs have also been coupled with various analytical instruments such as GC-MS, LC-MS/MS and HPLC. All MTs have offered good recoveries and lower LODs for drugs of abuse, which is a crucial requirement in drugs of abuse analysis. Moreover, amount of sample required to perform extraction is significantly less in comparison to LLE and SPE.

Applications of other techniques such as vortex-assisted liquid-liquid microextraction, ionic liquid based DLLME, single drop microextraction etc. may also be demonstrated and compared to each other in order to get high yield and sensitive extraction technique for forensic drug analysis. Additionally, MTs may also be applied for analysis of other classes of drugs of abuse such as benzodiazepines, barbiturate and anti-depressants.

Acknowledgments

Author is thankful to Coordinator, Central Forensic Science Laboratory, Directorate of Forensic Science Services, Ministry of Home Affairs, Guwahati for his constant support and guidance throughout the preparation of this manuscript.

Conflicts of interest

There are no financial or other relations that could lead to conflicts of interest.

References

1. Elmongly H, Rehim MA. Saliva as an alternative specimen to plasma for drug bioanalysis: A review. *Trends in Analytical Chemistry*. 2016;83:70–79.
2. Schramm W, Smith RH, Craig PA, et al. Drugs of abuse in saliva: A review. *J Anal Toxicol*. 1992;16(1):1–9.
3. Spiehler V. *Drugs in saliva, Clarke's Analysis of drugs and poison*. 3rd edn. Phamaceutical Press, UK. 2004.
4. Jain R, Singh R. Applications of dispersive liquid–liquid microextraction in forensic toxicology. *Trends in Analytical Chemistry* 2016;75:227–237.
5. Jain R, Singh R, Sudhaker S, et al. Coupling microextraction with thin layer chromatography–image processing analysis: A new analytical platform for drug analysis. *Toxicology and Forensic Medicine Open Journal*. 2017;2(1):17–25.
6. Jain R, Singh R. Microextraction techniques for analysis of cannabinoids. *Trends in Analytical Chemistry*. 2016;80: 156–166.
7. Samanidou V, Kovatsi L, Fragou D, et al. Novel strategies for sample preparation in forensic toxicology. *Bioanalysis*. 2011;17(3): 2019–2046.
8. Pragst F. Application of solid–phase microextraction in analytical toxicology. *Analytical and Bioanalytical Chemistry*. 2007;388(7): 1393–1414.
9. Cody JT. *Amphetamines: methods of forensic analysis. Handbook of Forensic Drug Analysis*. Elsevier Academic Press, USA. 2005.
10. Yonamine M, Tawil N, Moreau RLDM, et al. Solid–phase micro–extraction–gas chromatography–mass spectrometry and headspace–gas chromatography of tetrahydrocannabinol, amphetamine, methamphetamine, cocaine and ethanol in saliva samples. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2003;789(1):73–78.
11. Gentili S, Torresi A, Marsili R, et al. Simultaneous detection of amphetamine–like drugs with headspace solid–phase microextraction and gas chromatography–mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2002;780(1):183–192.
12. Meng P, Wang Y. Small volume liquid extraction of amphetamine in saliva. *Forensic Science International*. 2010;197(1–3):80–84.
13. Montesano C, Simeoni MC, Curini R, et al. Determination of illicit drugs and metabolites in oral fluid by microextraction on packed sorbent coupled with LC–MS/MS. *Anal Bioanal Chem*. 2015;407(13):3647–3658.
14. Hall BJ, Doerr MS, Parikh AR, et al. Determination of cannabinoids in water and human saliva by solid–phase microextraction and quadrupole ion trap gas chromatography/mass spectrometry. *Anal Chem*. 1998;70(9):1788–1796.
15. Fucci N, Giovanni ND, Chiarotti M, et al. SPME–GC analysis of THC in saliva samples collected with "EPITOPE" device. *Forensic Sci Int*. 2001;119(3):318–321.
16. Luo D, Chen F, Xiao K, et al. Rapid determination of Δ9–tetrahydrocannabinol in saliva by polymer monolithic microextraction combined with gas chromatography–mass spectrometry. *Talanta*. 2009;77(5):1701–1706.
17. Jager LD, Andrews ARJ. Development of a screening method for cocaine and cocaine metabolites in saliva using hollow fiber membrane solvent microextraction. *Analytical Chimica Acta*. 2002;458(6):311–320.
18. Fucci N, Giovanni ND, Chiarotti M. Simultaneous detection of some drugs of abuse in saliva samples by SPME technique. *Forensic Sci Int*. 2003;134(1):40–45.
19. dos Santos Lucas AC, Bermejo A, Fernández P, et al. Solid–phase microextraction in the determination of methadone in human saliva by gas chromatography–mass spectrometry. *J Anal Toxicol*. 2000;24(2):93–96.
20. Ranjbari E, Azizi AAG, Hadjmohammadi MR. Preconcentration of trace amount of methadone in human urine, plasma, saliva and sweat samples using dispersive liquid–liquid microextraction followed by high performance liquid chromatography. *Talanta*. 2012;94: 116–122.
21. Curini R, D'Ascenzo G, Del Carlo M, et al. Micro–solid phase extraction coupled with high–performance liquid chromatography–tandem mass spectrometry for the determination of stimulant, hallucinogens, ketamine and phencyclidine in oral fluids. *Anal Chim Acta*. 2010;675(2):132–137.