

DETERMINATION OF COCAINE IN SPIKED SALIVA USING LIQUID-LIQUID
EXTRACTION AND GAS CHROMATOGRAPHY- MASS SELECTIVE
DETECTOR METHODS.

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DEDICATION

I dedicate this work specially to my beloved late father Abdul Latheef Ali, my mother Khaleela Hussain ,my wife Khadeeja Mohamed and my daughters Iram and Iraj.

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ABSTRACT

Over the past few years, there have been a rapid development in forensic drug testing in biological specimen. Urine and blood are the most commonly used biological matrices for detection of cocaine. However, there are some disadvantages such as invasive to the privacy and personal safety of the donor as well as the instability of the cocaine in that biological matrix. Therefore, in this study, saliva has been proposed as an alternative matrix since the collection of saliva is non-invasive and rapid. In this study, the liquid-liquid extraction (LLE) method was utilized to extract cocaine from saliva before the analysis was done using optimized conditions of gas chromatography and mass selective detector (GC-MSD). Diethylether: isopropanol with volume to solvent ratio 3:5 was used in this study. The optimized method was validated by evaluating calibration curve, linearity, limit of detection (LOD), limit of quantification (LOQ), percentage recovery and intra-assay and inter-assay precision accuracy. From the calibration curve (0.5-10 ppm), good coefficient of determination was obtained at $r^2 = 0.9994$. LOD and LOQ were obtained at 0.29 and 0.97 ppm respectively. From the precision and accuracy analysis of intra and inter-day, lower coefficient variance (intra: 1.96%; inter: 0.79%) and high accuracy were attained (intra: 102.33%; inter: 101.20%). A mean recovery of 85.20% with acceptable coefficient of variance ($CV < 6\%$) were also acquired. Finally, the stability of cocaine in the saliva were studied for 13 days. The analysis found that cocaine can be recovered with a mean recovery $> 70\%$ without addition of any preservatives or buffers. The study showed that the analysis of cocaine in saliva is robust, hence it can be used as an alternative matrix for forensic drug testing.

ABSTRAK

Sejak beberapa tahun kebelakangan ini terdapat perkembangan yang pesat di dalam ujian forensik untuk pengesanan dadah menggunakan spesimen biologi. Air kencing dan darah adalah contoh matrik biologi yang paling biasa digunakan untuk pengesanan dadah jenis kokain. Walau bagaimanapun, terdapat beberapa kelemahan seperti pencerobohan kepada privasi dan keselamatan serta ketidakstabilan kokain di dalam matriks biologi tersebut. Oleh itu, dalam kajian ini, spesimen air liur telah dicadangkan sebagai matriks alternatif kerana pengambilan sampel air liur adalah tidak invasif dan cepat. Dalam kajian ini, kaedah pengekstrakan cecair-cecair telah digunakan untuk mengekstrak kokain dari air liur sebelum analisis dilakukan menggunakan kaedah kromatografi gas spektrometri jisim (GC-MSD) yang telah dioptimumkan. Dietil ether: isopropanol dengan nisbah isipadu pelarut 3:5 telah digunakan dalam kajian ini. Kaedah yang optimum ini telah disahkan dengan menilai parameter seperti kelinearan, keluk penentukuran, had pengesanan (LOD), had anggaran kuantiti (LOQ), peratusan pemulihan, serta ketepatan (precision accuracy) intra-cerakin dan inter-cerakin. Daripada penentukuran lengkung (0.5-10 ppm), pekali penentuan (r^2) yang baik diperolehi dengan kelinearan adalah $r^2 = 0.9994$. Untuk had pengesanan (LOD) dan had anggaran kuantiti (LOQ), masing-masing diperolehi pada 0.29 ppm dan 0.97 ppm. Sementara itu, bagi analisis ketepatan intra-cerakin dan inter-cerakin, pekali variasi yang paling rendah (intra: 1.96%; inter: 0.79%) dan ketepatan yang paling tinggi telah dicapai (intra: 102.33%; inter: 101.20%). Purata peratus pemulihan adalah 85.20% dengan pekali variasi yang boleh terima ($CV < 6\%$) juga diperolehi. Akhir sekali, kestabilan kokain di dalam air liur telah dikaji selama 13 hari. Analisis telah membuktikan bahawa kesan dadah jenis kokain boleh dipulihkan dengan purata pemulihan $> 70\%$ tanpa penambahan sebarang pengawet atau buffer. Kajian ini menunjukkan bahawa analisis dadah jenis kokain di dalam air liur adalah sangat berkesan dan ia boleh digunakan sebagai matriks alternatif untuk ujian forensik dadah.

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LIST OF ABBREVIATIONS

CE	-	Capillary Electrophoresis
CV	-	Coefficient of Variance
EME	-	Ecgonine Methyl Ester
EMIT	-	Enzyme Multiplied Immunoassay Technique
GC-MSD	-	Gas Chromatography-Mass Selective Detector
HPLC	-	High Performance Liquid Chromatography
IS	-	Internal Standard
LLE	-	Liquid-liquid extraction
LOD	-	Limit of Detection
LOQ	-	Limit of Quantitation
m/z	-	Mass to Charge
mL	-	Milliliter
mL/min		Milliliter per Minute
N/A	-	Not Applicable
ng/mL	-	Nanograms per Milliliter
ng/mg	-	Nanogram per Milligram
NCOC	-	Norcocaine
Ppm	-	Parts per Million
Ppb	-	Parts per Billion
r ²	-	Coefficient of Determination
t _R	-	Retention Time
RSD	-	Relative standard deviation
SD	-	Standard Deviation
SPE	-	Solid Phase Extraction
SPME	-	Solid Phase Microextraction
TLC	-	Thin Layer Chromatography
UV	-	Ultra Violet

LIST OF SYMBOLS

\bar{x}	-	Mean
$^{\circ}\text{C}$	-	Degree Celsius
$<$	-	Less than
$>$	-	Greater than
\pm	-	Plus or Minus
$=$	-	Equal to
$\&$	-	And
μl	-	Micro Litres
μm	-	Micro Meters
$\%$	-	Percent

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Cocaine is a tropane alkaloid that produce powerful stimulating effects on the central nervous system. Once administered into the body, it increases dopamine in the brain by self-consciousness of addiction (Bhatia *et al.*, 2017). Dopamine is an endogenous neurotransmitter that controls movement, critically for cocaine, the reward pathway making the drug highly addictive (Adinoff, 2004). It has local anesthetic properties and even today it is used within the ears, nose, throat (ENT) practice to reduce pain in nose, throat and eye surgeries. However, its medical practice adoption has been decreasing as lower addictive alternatives (e.g. barbiturates, benzodiazepine) have become available (Johnson, 2010). Besides its medical practicality, it is also used as a recreational drug around the world

Cocaine is mostly inhaled (insufflation) or injected (intravenous) but oral intake is also practiced sporadically (NIDA, 2016). When inhaled, the vapour passes through the lungs and is absorbed into the bloodstream. When injecting, cocaine is dissolved in water and then administered intravenously causing the drug to enter directly to the bloodstream before producing high stimulating effect. When it is snorted, it passes through the nostrils onto the bare nasal tissues and gets absorbed into the bloodstream. Besides of insufflation and intravenous, cocaine is also rubbed on the gums for oral administration.

In common with other abused drugs, cocaine has the characteristics of dependency, withdrawal symptoms and tolerance (MacLaren, 2016). It can induce serious and debilitating psychological and physiological change. Psychological risks include unpredictable behavior, the tendency towards violence, loss of appetite, loss of sex drive, depression, paranoia, confusion, anxiety and losing touch with reality.

Physiological risks include the increase in blood pressure, heart rate and body temperature, brain seizures, hepatitis or acquired immune deficiency syndrome (AIDS) when needles are shared and decreased capacity to combat diseases (NIDA, 2016).

Determination of parent drug from biological matrices is one of the major concerns. The instability of cocaine in biological matrices makes the cocaine to degrade to its derivatives (Yoshioka & Stella, 2007). The amphoteric behavior of cocaine is highly dependent on the pH in biological matrices makes challenging for forensic analysis in toxicology (Baselt, 2004). Since blood and urine required intensive sample preservation and preparation. However, specific study of cocaine in saliva is limited. Therefore, this study explores the potential of saliva as sample matrix.

Solid phase extraction (SPE) is proposed method for analysis of cocaine in biological fluids (Farina *et al.*, 2002). SPE avoids sample loss through emulsion formation during extraction and provides clean extracts however, it is expensive and may require derivatization (Valente *et al.*, 2012). In this study, LLE method was chosen as it provides simple, rapid and low cost for the analysis of cocaine in saliva. Gas chromatography- mass selective detector (GC-MSD) instrument was chosen for its versatility for drug analysis.

1.2 Problem Statement

Drug addiction is a major threat to all societies in every country in almost every single way. It endangers to major health problems, to family as well as to the community. Crimes such as drugged driving, violence, child abuse and work problems also arise due to abusive drugs. According to statistics reported by Substance Abuse Mental Health Service Administration in 2013 (SAMHSA, 2013), among the total population of the United States (aged 12 or above), 1.5 million (i.e. 0.1%) people are cocaine abusers in all forms.

Throughout recent years in history, several methods of drug testing have been introduced. Though the testing methods have been advanced, the collection and preservation of samples is very challenging. Despite the most generally used specimen for drug testing is urine, (Leyton *et al.*, 2011; Marchei *et al.*, 2008), it has drawbacks like specific requirement for storage and low window of detection. The urine specimen must be kept at a certain temperature to prevent bacteria forming which will alter the result. The collection of specimens will invade privacy of the donor and must be required under observation to maintain integrity of sample (Valente *et al.*, 2012). During donation of the urine sample, the corresponding gender of the donor must be attended to observe during sample collection. This is to protect gender privacy, avoid adulteration and sample exchange.

Blood is another sample matrix; however, blood samples must be handled carefully to prevent the hemolization. In matrix such as plasma and whole blood, cocaine can easily hydrolyze to its derivatives at room temperature (Sun *et al.*, 2002). Collection of blood is also invasive technique which requires preventive health measure while handling the specimens. Certain precautions must be taken by the collection personnel to prevent infection from of diseases.

Currently in Maldives, Enzyme Multiplied Immuno Assay Technique (EMIT) is being used to analyze cocaine and other abusive drugs in urine and plasma. Immuno assay technique has become an essential part in screening for abusive drug in forensics. Despite many advantages of EMIT such as no pretreatment of samples and rapid results, there are drawbacks like false negative results, careful interpretation and less sensitivity as compared to GC-MSD. Hence for definite qualitative and quantitative analysis, other techniques such gas chromatography, liquid chromatography or capillary electrophoresis are highly recommended.

In Maldives, Drug and Chemical Laboratory of Maldives Police Service have made a stand to shorten the total duration for the analysis and issuance of analysis report within 5 days to compensate the timeline for the investigation (Rameez, 2015). And also, prosecution of detainees will get a maximum period of 30 days in criminal procedure code article 94 of 12/2016 of Maldives. Therefore, the evidence requires

shorter duration and immediate analysis, hence the study also investigate the stability of cocaine in the duration of 13 days.

1.3 Objectives

The objectives of the study are as follows:

- i. To perform qualitative and quantitative analysis of cocaine using gas chromatography and mass selective detector (GC-MSD).
- ii. To optimize the method of extraction of cocaine from (saliva) using liquid-liquid extraction (LLE).
- iii. To study the stability of cocaine in saliva.

1.4 Scope of the Study

This research was conducted using drug standards of cocaine and codeine purchased Lipomed (USA) company. Drug free saliva of a subject was used as a biological matrix. Codeine drug standard was used as an internal standard suggested by Yilmaz *et al.*, (2017).

Known concentration of cocaine was spiked into drug free saliva, which was then extracted using LLE. The recovery of cocaine from extracted samples was analyzed and quantified using GC-MSD. The responses of cocaine and codeine were detected using the method adopted from the journal previous researcher Yilmaz *et al.*, (2017). The experiment was repeated with triplicates and the average response of peak area ratio (cocaine is to internal standard). Standard deviation, slope, limit of detection and limit of quantification was then calculated. Reproducibility and repeatability were conducted by performing intra-day and inter-day analysis before relative standard deviation was calculated to measure precision and accuracy of the method. LLE

method was optimized for determining the cocaine in saliva. Further, Stability of cocaine for consecutive days were then studied.

1.5 Significance of the Study

The current study will benefit us to determine the cocaine from saliva as compared to blood and urine since the extraction method needed is simple. Selection of saliva as the sample matrices required non-invasive sampling techniques which can prevent any infection or accidental injuries during the sampling process. The sampling method also requires less privacy invasive whereby the observation during the sampling process can be done without the need of a close setup room. Moreover, since the extraction method requires simple sample preparation, the analysis can be done in more rapid manner. The optimized method then will be useful for forensic investigation (i.e. Maldives Police Service, Royal Malaysian Police, Jabatan Kimia Malaysia) especially for cases involving the abuse of cocaine.

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