ForensicAsia
THE ASIAN FORENSIC SCIENCES NETWORK NEWSLETTER | ISSUE 13 | 2023

AFSN President's Address

Dear colleagues and friends,

Greetings to all colleagues in Asia and around the world!

I am very pleased to share with you all about our recent endeavors to improve Forensic Science in this part of the Asia-Pacific region. This is an exciting time for our organization, as we continue to establish ourselves as leaders in the field and make strides toward advancing forensic science.

As we move forward, we desire for a more innovative and inclusive forensic community in the Asian Forensic Sciences Network through Information and Communications Technologies Development. Thus, the AFSN will be the avenue for the ten strong workgroups to orchestrate robust discussions and productive activities throughout the calendar year using interactive platforms such as WHOVA, ZOOM, MICROSOFT TEAMS, GOOGLE MEET, and many others.

The leadership of AFSN collectively stands to take the opportunity of an evolving society to redesign policies and practices and ensure that all member institutes and individuals are empowered to their full extent. Therefore, it is imperative that we continue to convene and interact in as many ways as possible. We will do this by harnessing the widely available digital platforms to conduct training, seminars, small group discussions, research, and meetings.

The Network has come a long way since its inception but there is still much work to be done. I, together with the members of the Board, implore each of you for your continued support and active participation; to remain committed and rally strong with our fellow forensic scientists as we realize the objectives of the AFSN in the post pandemic world.

Our Network will advocate a new standard of inclusivity and professional cohesion among personnel of the criminal justice system of diverse culture and background. Together, we will usher the Asian Forensic Sciences Network to the next level of interconnectivity and productivity.

Our journal, the ForensicAsia, is an essential vehicle for sharing knowledge and highlighting new advancements in forensic science. As such, we are committed to making it one of the best resources available to our colleagues, the broader scientific community, and the public.

We are fortunate to have received many high-quality submissions from a broad range of disciplines, and our editorial team has done an excellent job in selecting and publishing articles and research that advances forensic science.

I would also like to recognize the hard work of our journal's editorial board led by Dr. Lui Chi Pang, and his team on the continued success of ForensicAsia.

Your commitment to highlighting the latest advancements and breakthroughs in forensic science is truly commendable, and I am continually impressed by the quality and depth of the content that you produce. Your efforts play a vital role in advancing the field of forensic science and helping to ensure that justice is served.

I look forward to future issues and encourage all members to continue submitting their work for publication.

Thank you for your support and commitment to advancing forensic science.

PBGEN CONSTANCIO T CHINAYOG JR
AFSN President
PNP Forensic Group
Philiipines

Editor's Address

Dear colleagues and members of AFSN,

First of all, let us welcome our newly elected AFSN President, Pol. Brigadier General Constancio Chinayog Jr. Under the new leadership, we have indeed had a fruitful year for our members with many activities being organised, including webinars and workshops since the beginning of 2023. As we are looking forward to our upcoming 15th AFSN Annual Meeting and Symposium in Kuala Lumpur, Malaysia, this September, I am also glad to see that we have received a variety of research articles for publication in our ForensicAsia.

In this Issue, we have a total of 9 technical articles and 3 case studies. They span across a wide range of disciplines, including toxicology, illicit drugs, questioned documents, crime scene investigation, fires & explosions, forensic biology, forensic anthropology and forensic science management. In addition, we are very glad to have received one article under the international scene from Prof. David Gidley of Forensics Worldwide P/L, two members' news from National Forensic Service (Korea) and Philippine National Police (Philippines), and one article from our new member SJS Institute of Forensic Science & Medicine (Korea) to introduce their institute.

Once again, I would like to take this opportunity to thank those who have supported ForensicAsia by sharing your valuable research and studies, our guest editors in reviewing the articles, as well as our editorial assistants who have assisted in the administrative matters and the artwork design for the online publication of this new Issue.

Happy reading and see you in Kuala Lumpur!

Dr Lui Chi Pang
Editor

Editorial Committee

For enquiries, feedback or contribution of articles, please email to asianforensic@outlook.com.
For contribution of articles, please refer to the guidelines at www.asianforensic.net.

Content

Content

AFSN Webinar Series "Titbits from around the world" Topic: Coping with backlog

Ms Nellie Cheng
Health Sciences Authority, Singapore
Email: nellie_cheng@hsa.gov.sg

Anticlockwise from top right: Ms Ranee Ho, Mr Barry Fisher, Mr Scott Oulton and Dr Angeline Yap addressing questions from the participants during the Question-and-Answer session.

AFSN launched the AFSN Webinar Series "**Titbits from around the world**" as an initiative to enhance the knowledge of forensic practitioners in Asia through inviting international forensic experts to share their knowledge and experiences in various issues and challenges facing the forensic laboratories. The title of the webinar series "**Titbits from around the world**" informs that the talks are intended to be short and succinct, just like small snacks, light but sufficient to satisfy the hunger for the moment, and tasty to delight our taste buds to long for more.

The Health Sciences Authority, Singapore hosted the first webinar of the Series on April 28, 2023 in virtual format. The topic "**Coping with backlog**" was chosen as having forensic casework backlog is a common challenge that many laboratories are facing. A total of 420 colleagues from 23 AFSN member institutes of 13 countries registered for the talk. Many colleagues also attended the webinar jointly as a group.

Mr Scott R Oulton (Deputy Assistant Administrator, Drug Enforcement Administration Office of Forensic Sciences, USA), Ms Ranee Ho (Director of Laboratories, Onondaga County Center for Forensic Sciences, USA) and Mr Barry Fisher (Past President of AAFS, IAFS, ASCLD) shared their invaluable experiences and strategies on how laboratories could tackle the common challenge by formulating policies, refining workflows, and changing team culture. Their talks were well received with 100% of the attendees finding the presentations beneficial and interesting.

AFSN would like to extend our sincere appreciation to Mr Oulton, Ms Ho and Mr Fisher for speaking in our webinar amidst their busy schedules. A special thanks to Mr Mike Cariola of ASCLD for introducing great speakers for the webinar.

Statistics of the webinar at a glance.

A group of officers from the Philippine National Police Forensic Group attending the Webinar.

Forensic Medicine Practioners – Virtual Case Conference

Joseph C. Palmero, MD (Chair, FMWG)
Philippine National Police, Philippines
Email: josephpalmero@gmail.com

The Forensic Medicine Workgroup (FMWG) held its first virtual case conference for 2023 dubbed as "Bring Your Case Forum" on March 29, 2023, at 5:00-6:00 PM (GMT +8). Dr. Goeun Lee of the National Forensic Service (South Korea) presented an unusual case of suicide by ligature of the chest, while Dr. Ahmad Hafizam bin Hasmi from the National Forensic Medicine Institute of Malaysia presented a case of traumatic brain injury due to an assault, with concomitant autopsy findings of severe coronary artery occlusion (heart attack). It was then followed by a question and answer portion.

There were 93 attendees from Malaysia, Indonesia, Thailand, Vietnam, South Korea, Jordan, and the Philippines. AFSN President BGen. Constancio T. Chinayog, Jr. also attended and witnessed the said event.

The purpose of the virtual forum is to have an avenue for forensic medicine specialists in Asia to share and discuss their unusual and interesting medicolegal cases.

Forensic Science education and field trip for Mongolian forensic experts and Thai students at the National Forensic Service

*Mr. Kyu-sun Shim, Dr. Dong Kye Lee, Dr. Nam Kyu Park**
National Forensic Service, Republic of Korea
Email: ok825@korea.kr

NFA, Mongolia, high-level staff.

NFA, Mongolian, forensic expert training.

Suan Sunandha Rajabhat University (SSRU) visit to NFS.

As the only forensic science organization in South Korea, the National Forensic Service (NFS) receives many visitors, such as, experts and students domestically and internationally. In the first quarter of this year, we provided training and visiting program to forensic experts from the National Forensic Agency (NFA) in Mongolia and forensic department staff and students from Suan Sunandha Rajabhat University (SSRU) in Thailand.

The training of the Forensic Experts of the Mongolian NFA was part of the Official Development Assistance (ODA) Project by the Korean NFS with the support of the Korea International Cooperation Agency (KOICA). It aimed to strengthen the capacity of the Mongolian NFA in the field of forensic science.

The training was being provided to seven experts in genetic, narcotics, and digital forensics for seven weeks, from March 6 to April 21, 2023.

In addition, from March 22 to March 24, a group of high-level officials from the Mongolian NFA visited NFS to learn about the electronically built forensic information portal system to strengthen evidence capacity.

President of NFS, Dr Park Nam Kyu, warmly welcomed the high-ranking officials and experts from the Mongolian NFA and gave a special lecture on Korean forensic science.

Staff and students from the Forensic Department of SSRU in Thailand visited the NFS on April 6, 2023, and were introduced to NFS toxicology/drug sector as well as fire/safety accident sector. Since signing a business agreement with the Central Institute of Forensic Science (CIFS), Thailand, in 2019, NFS Korea has continuously exchanged with CIFS to promote the exchange of forensic science knowledge and experience between Korea and Thailand. As part of this connection, NFS Korea actively supported the SSRU visit, which provided an opportunity to introduce Korean forensic science to the students.

Our Duties - SJS Institute of Forensic Science & Medicine

*Dr Joong-seok seo, Dr Young-shik Choi, Ms Eun-yeong Kim**
SJS Institute of Forensic Science & Medicine, Republic of Korea
Email: admin@sjsforensic.com

SJS Institute of Forensic Science & Medicine (SJS Institute) established in Oct. 2018, has been providing forensic medicine service (autopsies and trauma diagnosis) and exporting Korean forensics facility to overseas. Do you remember "*New horizon of forensic science*" that was the slogan of World Forensic Festival at Seoul in 2014? Our experts are working for improvement and development of forensic science system to response the atrocious crimes, new social issues including Gender Based Violence (GBV) and narcotic problems in the world. We would like to introduce our works to members of AFSN.

Firstly, the key persons of SJS institute are Dr Joong-seok Seo (2nd president of NFS, 4th President of AFSN) and Dr Young-shik Choi (3rd president of NFS), who are briskly working as an emeritus medical examiner of National Forensic Service, Korea. They are performing autopsy twice a week and teaching forensic medicine to students nowadays. In addition, they are working as consultants of forensic medicine at Korean Police Agency, Korean Prosecutor's Office and Supreme court.

In Korea, Official Development Assistance programs have supported the enhancement of capacity in field of criminal justice system and forensic science to developing countries. Those are known for KOICA (grant) and EDCF project (loan). For example, based on KOICA funds, NFS had firstly performed "capacity project on the enhancement of forensic science at GAD in Sri Lanka" from 2014 to 2017. And several programs were consecutively performed to Asian countries. SJS Institute works for planning and consulting of the new projects with KOICA and Korean EXIM bank for providing Korean Forensic Medicine & Science facilities, including training, equipment, construction of laboratory and autopsy room, and IT system with LIMS, to developing countries in the world.

Through the feasibility study, we offer the best solution how to supply forensic facility customized for the developing countries. As a result, our Institute have accomplished two projects: feasibility study of modernization project of National Institute in Mongolia and feasibility study of capacity project on building of forensic medicine facility for response GBV in Bolivia.

Our aim is the share of the far-advanced Korean forensic system for crime-free world. We hope our technology and knowledge are widely used with members of AFSN for development of forensic science and medicine. We look forward to freely communicating and cooperating for a better future.

As medical examiner, Dr Seo and Dr Choi Perform Autopsy on every week.

We have promoted ODA project for establishing forensic medicine & forensic science for developing country.

We attended pre-feasibility study for building on the forensic science for response GBV in Bolivia in 2022 by KOICA.

ICITAP Indonesia - Forensics Development Project

Prof David Gidley
Forensics Worldwide P/L, Indonesia & Australia
Email: gidleyd46@gmail.com

ICITAP is the acronym for the International Criminal Investigative Training Assistance Program, a law enforcement aid program delivered under the auspices of the US Government (USG), Department of Justice (DoJ). The ICITAP Indonesia Forensics Development Project (FDP) commenced in 2005 has ceased due to funding cuts recently. Initially a Forensic Assistance Pilot Project (FAPP), in Surabaya, East Java, it assessed the capacity building needs of the Indonesian National Police (INP), Forensic Laboratory system (Labfors).

FAPP developed into a national project after early successes in Surabaya including the introduction of digital photography for crime scene and evidence recording, upgrading of ballistics casework using a Leica FSC comparison microscope/databasing system (complete with bullet recovery tank), donating a VSC 5000 questioned document comparator, and many laboratory practise enhancements. These ICITAP funded donations were soon replicated across the (then) seven Labfor systems, providing an excellent return on investment (ROI) on the initial USG/DoJ donations.

A key finding from the FAPP assessment was the need for modern forensic biology capacity, taking them from routine ABO serological testing to DNA analysis. 2009 saw the official opening of a turn-key forensic DNA facility which included building a DNA suite, total DNA equipment package and even two new DNA staff, with molecular biology qualifications. This was the first forensic DNA facility within the Labfor system and went on to provide investigative breakthroughs in many cases where no other linking evidence was available. The remarkable value of DNA technology; which saw this facility later replicated by the INP at the Central INP Forensic Laboratory (Puslabfor) in Jakarta.

All of these equipment donations were supported with targeted training as an integral part of their full commissioning, with this training formally recorded in each individuals' training record, and with each instrument having a dedicated logbook, the first entry in which was the successful commissioning and certified operation of that instrument. Both these activities serving as a focus/key part of the base for the future goal of developing a comprehensive quality management system from which to seek international accreditation.

Another essential part of this forensic capacity building was ensuring the Labfors had adequate facilities in terms of security and safety. Security involved the provision of perimeter fences, electronically controlled entrances, and strategic CCTV installations, monitored centrally at each Labfor's Security Post. Clearly security is also an element in Labfor safety relating to evidence integrity and staff well-being, and safety was further supported with facility enhancements including chemical safety cabinets, fume cupboards, safety stations and fire extinguishers. Evacuation plans and appointed Safety Officers completed this package.

Once the solid operational base had been established in all seven Labfors, attention was turned to fully documenting their entire forensic laboratory operations. This was achieved through the development of a Laboratory (Quality) Manual containing all relevant information for staff (especially for new staff orientation). Much of this information was presented in detail as it directly pertained to the Labfors, and other information of a corporate nature (home organization policies and procedures), was summarized and "pointed to" clearly, by specific references to other INP documents/manuals. The Lab Manual was supported with Method Manuals for each Work Unit and each Method Manual had a directly related Training Manual, providing a structured and consistent learning approach including clear assessment steps, at the successful completion of which the trainee could be formally authorized as competent to undertake casework using those Methods. Each Work Unit also had a Technical Procedures Manual, including Casework Forms and safety issues for that Unit, which spelled out day-to-day operational requirements of the Unit, so that staff within had clear and unambiguous SOP's to follow. All Manuals were structured to meet ISO standards and also importantly to satisfy INP organizational formats and standards.

While this was initially a huge amount of work to produce the Manuals set, it served as a superb base for quality forensic services provision, and once in place was easier to maintain and improve going forward with Annual Management Reviews, and after each accreditation assessment. This continuous improvement cycle is one of the major benefits of seeking ISO/IEC 17025:2017 and/or ISO/IEC 17020 accreditation, along with the boost to forensic scientist ability and confidence at scenes, in the laboratory and while providing expert evidence to Courts. All original seven INP Labfors are now fully accredited.

There are now 11 INP Labfors, and further work is still needed to develop an Indonesian Criminal DNA Database and create a Forensic Intelligence capability to proactively support investigations. Alas this cannot be undertaken by ICITAP, but it remains that FDP has delivered a paradigm shift in Indonesia away from confessional evidence to objective scientific evidence, greatly strengthening the Indonesian justice system.

I will now continue my forensic consulting through Forensics Worldwide P/L for any assistance AFSN members may require.

Colour-coded Manuals we developed with the INP Labfor Directors, Quality Managers and staff.

Map of the geographic service areas of the now11 Indonesian National Police (INP) forensic laboratories (Labfors).

Interpretation of Dynamic Activity from Analysis of Static Footwear Impressions

[1] Prof Huang Yu [2]Dr He Huisuo [3]Mr Cai Shaoguang*
[1]China People's Police University (CPPU), School of postgraduate, China.
[2]Longquanyi District Branch, Chengdu Public Security Bureau, China.
[3]China People's Police University (CPPU), School of Security, China.
The People's Republic of China
*Email: shaoguangcaicai@163.com

Abstract

Footwear impressions, as one of the most common physical evidence, are widely present in many crime scenes. It is pervasive, easy to find and extract, and can effectively reflect the characteristics of the crime process. Through the static footwear impressions analysis and research, the static footwear impressions is interpreted dynamically in terms of activity, which can effectively provide clues and directions for solving cases.

Key words: footwear impressions, evidence, static, analysis and research, dynamic.

According to statistics, in 2017, the national public security authorities investigated 5.316 million scenes of various crime cases[1], of which the Deoxyribo Nucleic Acid(DNA) extraction rate was 12.48% and the fingerprint extraction rate was 7.75%. The footwear impressions extraction rate was 13.22% (Table 1). Statistically, among the various types of trace physical evidence at the scene,the footwear impressions have the most left behind, the highest occurrence rate, the highest incidence rate and the highest extraction rate.

Classification	Extraction Numbers	Extraction Rate
Deoxyribo Nucleic Acid	663,400	12.48%
Fingerprint	412,000	7.75%
Footwear impressions	702,800	13.22%

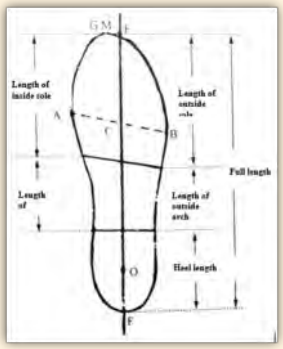
Table 1

Footwear impressions, as one of the most common traces of physical evidence, are widely present in many cases scenes. It is pervasive, easy to find, extract, and can effectively reflect the characteristics of the crime process. Compared to fingerprints and other traces of physical evidence, footwear impressions are difficult to disguise, eliminate, but easy to extract traces of physical evidence in the scene investigation[2]. How to make full use of the footwear impressions traces to obtain clues, provide the direction of investigation and solve cases?

At this stage, there are various methods for the analysis of footwear impressions in crime scene investigation and examination.

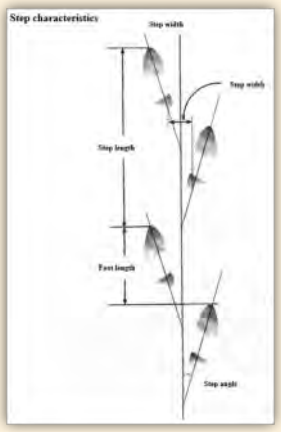
Single footwear impressions measurement and analysis

Human balance is the balance relationship (dynamic stereotype) formed by long-term walking or training, that is, this balance relationship determines the particular walking posture of each person [3]. Through the particular walking posture, it is possible to analyze the morphological characteristics and structure of the shoe print, the wear characteristics of the sole, and the dynamic morphology, thus providing guidance for the case.



Measurement and analysis of adult in series of footwear impressions

The particular walking posture of each person can be analyzed for the gait characteristics of the adult footwear impressions, limbs movement characteristics, and thus advance the case.



Step characteristics.

Analysis of footwear impressions in combination with other technical tools.

1 Combined with physical and chemical testing, Deoxyribo Nucleic Acid(DNA) testing, reduce the number of possible suspect's actions by the soil and trace elements that left in the Footwear impressions of the scene.

2 Combined with microbiological testing: to confirm the suspect's scope and time of action, etc. through the microorganisms in the footwear impressions of the scene.

3 Combined with video investigation: through the action trajectory of the footwear impressions determine the route of the suspect's travel and find out (identify) the suspect.

4 Combined with video inspection: The action track of the footwear impressions is combined with examination of CCTV evidence to identify suspects.

At this stage: the identification methods of in crime scene investigation and inspection.

The identity of gait

Combining the information of step length, width and angle of a single footwear impression and a series of footwear impressions, or the information of gait and wear characteristics of single footwear impressions reaction can profile the person, gender, height, age, physique and even psychological changes, etc., and portray personnel characteristics which includes but not limited to occupation, habit, even the characteristic of pretending, disablity.

Identification of the same kind of sole pattern

Through the identification of the characteristics of the sole pattern, it can effectively determine the number of suspects, can connect series of cases , and possibly be the breakthrough piece of evidence in a case.

Determination of the same sole pattern

Random individual features formed on the shoe are compared with the corresponding parts in the shoe print, to determine whether they are consistent and the reasons for the differences.

How footprints are made

Footprints are produced by the transformation of the actor's position moving in space. Human walking is done by the various parts of the brain that control walking movement. When the front foot steps forward, the body leans forward, the heel, midfoot, and ball of the back foot are raised in turn, leaving the ground, and the toes are forced back and down and leaving the ground, at the same time, the front foot lands on the ground, and the heel, midfoot, ball, and toe parts touch the ground in turn (rolling process), and the back foot steps forward to complete the walking movement reciprocatingly. With each step of the actor's movement, the shoe will come into contact with the bearing body, resulting in deformation between the sole and the bearing body, and the transfer of substances between the shoe and the bearing body, the substance of the shoe left on the object being stepped on, and the substance of the bearing body left on the insole. Since the actor is in direct contact with the bearing body under the effect of gravity, at each step, force interaction occurs between the insole of the shoe and the bearing body. Therefore, strictly speaking, every footwear impression has different traces left on the object because of the different actors, or the different objects that bear the trace, or the direction, angle, and force of the actor's landing. [4]

Footwear impressions are created mainly due to the pressure created by gravity on the supporting foot, i.e.

(P)ressure=(F)orce/(A)rea

It can be deduced according to the pressure formula, the heavier the pressure on the object per unit area, then the greater the pressure. In a crime scene, the pressure can again be simplified to

$$(\mathbf{F})\mathbf{orce} = \sum_{i=1}^{n} \mathbf{Fi}$$

Through the above pressure derivation formula, it can be deduced that in the case of a constant unit area, the pressures show a positive relationship, that is, if the individual pressures increases, the total pressure becomes larger; if the individual pressures decreases, the total pressure becomes smaller.

In the crime scene, because the suspect is moving, the route taken by the suspect may be identifiable by the footwear impression. Each footwear impression can be understood as the pressure of gravity acting on the unit area of the sole of the foot. If the pressure changes in different parts of the foot, for example, due to movement, then the footwear impression in that area may appear darker and with clearer edges. Correspondly, if the pressure is lighter, the shoe print may appear lighter and more faint.

During the actual scene investigation, numerous footwear impression are likely to be discovered. By analyzing these footwear impression, investigators can determine the movement of the suspect (s) in the crime scene. This analysis and subsequently interpretation using findings from footwear impression research can improve the efficiency of solving cases.

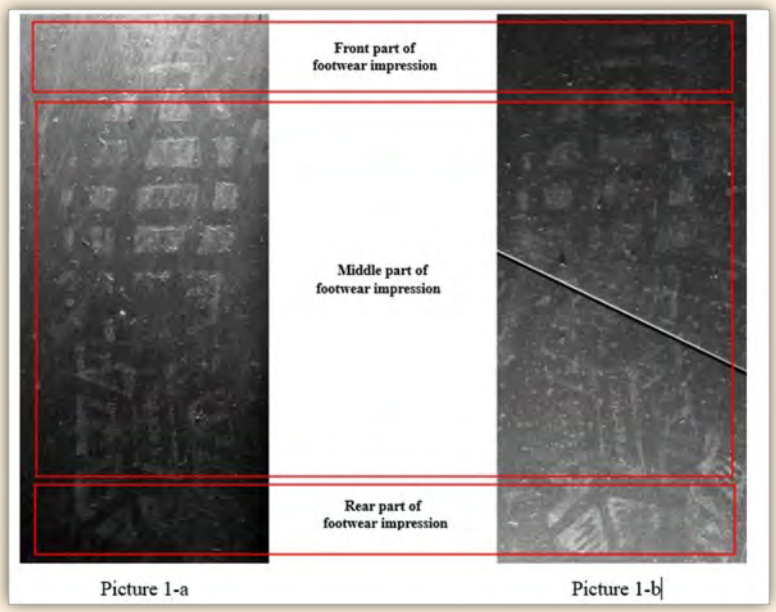
Case example

A case of theft occurred at a jewelry store. A total weight of 5 kilograms of gold jewelry had been stolen. The loss amounted to more than 2 million yuan. After investigation, investigators found that the suspect was wearing gloves to commit the crime, and therefore left no fingermarks. However, there were a large number of footwear impressions left at scene.

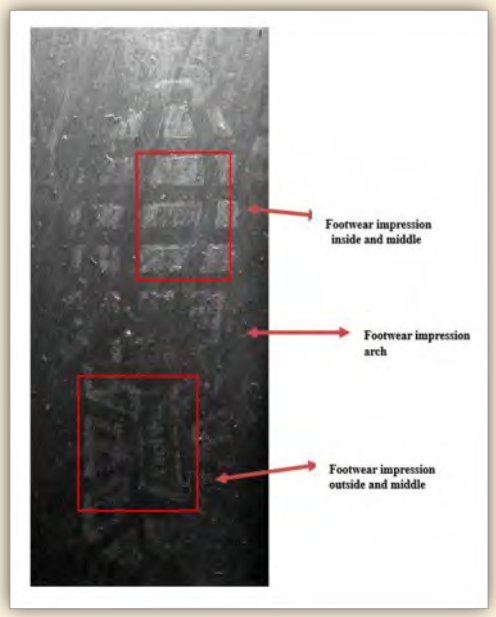
After laboratory testing and analysis of the footwear impressions, the crime was confirmed as a one-person operation and a suspect was identified. As the only evidence was the footwear impressions left the scene, it was necessary to get more information by analyzing and researching the footwear impressions.

The walking process can be, divided into three stages-starting, mid-stance, landing stage. Due to landing and starting stage being affected by individual subjective factors and object factors, so people investigate the process of the mid-stance stage [5]. The more stable part of the footwear impression is the middle, especially the areas with higher contact pressure. The front and the rear parts of the footwear impression are not stable.

As shown in the Picture 1, among the features of the middle of the footwear impression, the left picture of footwear impression (Picture 1-a) marks are darker and more solid, especially in the inner and middle of the forefoot, and the outer and middle of the heel area (Picture 2).



Picture 1



Picture 2= Picture 1-a

What is the reason for the change in the intensity of the footwear impression- i.e., the change in pressure?

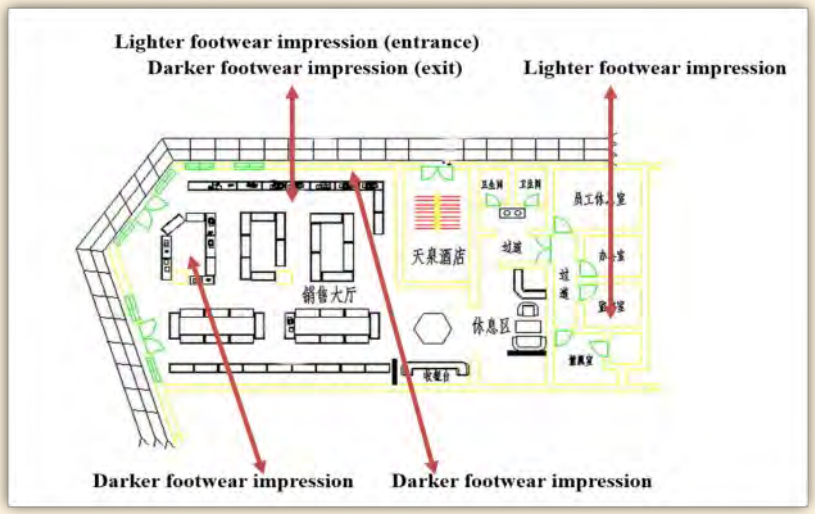
(P)ressure=(F)orce/(A)rea

$$(F)orce = \sum_{i=1}^{n} Fi$$

F_1= Suspect's own weight

F_2= Weight of stolen items

The reason for this difference in pressure was the change in weight. According to the findings of the case, it is understood that the reason for the change in weight is that the suspect was carrying stolen items such as gold and silver jewelry weighing up to 5kg (F_2),one-twelfth that of his own weight (F_1).The change in pressure was revealed through multiple footwear impression as the suspect was walking with the stolen goods on his back. According to the analysis of the Pictures 1, the left picture (Picture 1-a) is the footwear impression left by the weight of the suspect after stealing the property, and the right picture (Picture 1-b) is the footwear impression left by the suspect before stealing the property.



According to the analysis and research of the footwear impression left at the scene, two valuable clues were identified.

1. Based on the footwear impressions, the route of the suspect's travel was deduced, and the suspect's entrance and exit were determined, namely: the suspect entered the scene and before stealing the property, the pressure of the sole was small, and the footwear impression was shallow and light; after stealing the property, the suspect's shoe sole pressure becomes larger and the footwear impression was darker.
2. According to the scene, the left picture of footwear impressions (Picture 1-a) was extracted from the ground at the original place of jewelry in the store; the right picture of footwear impressions (Picture 1-b) was extracted from the ground in the store, of the remote surveillance room. This suggests that the suspect entered the store, and did not first steal the jewelries, but went to the store's monitoring room to cut off the surveillance cameras, and then went back to the store to steal property. The final judgment is that the suspect is quite familiar with the layout of the jewelry store, can accurately locate the surveillance room, and has a certain sense of anti-detection, avoiding the influence of indoor surveillance.

In the above case, the dynamic path of the suspect is shown through the analysis of the static footwear impressions, and important clues were derived, that helped the investigation. Of course, according to the dynamic analysis and judgment of the static footwear impressions left at the scene, we are able to further analyze the case, such as whether the suspect tried the crime scene beforehand, whether the suspect familiar with the crime scene, the suspect's path of travel combined with video analysis and other issues, which are not within the scope of this article.

Through the analysis and research of the static footwear impressions, the site investigator can explain difference in the static footwear impressions, which can provide direction to the investigation of the case and play a decisive role in the final outcome of the case.

Interpretation of dynamic activity from the analysis of the static footwear impressions, has a positive effect on identifying the movement of suspects involved in theft of heavy objects (e.g., theft of cable lines, transferring and transporting bodies in homicide cases, etc.). It aids in, determining the entrance and exit points in a crime scene, profiling the suspect's behavior and motive, and providing valuable clues.By understanding the nature of the case, this analysis can also provide direction for solving the cases.

References

[1] Li Xia. Comprehensively implementing the "One Officer & Four Must Be" to improve the level of scene investigation ability [N].China Police Daily,2018-03-26(006)

[2] Hu Nan, Wang Defei. Exploration of the importance of footwear impressionss and traces in detecting and solving cases [J]. Law Expo,2017(18):177.

[3] Han Junliang. Footwear impressions test identification [M] Beijing: Chinese People's Public Security University Press, 2008:60-87

[4] William J. Bodziak. Shoe print evidence: discovery, extraction, and examination (2nd ed.) [M] Beijing: Chinese People's Public Security University Press, 2015:1-25

[5] Sun G, Li Ynlong. Talking about the value of Footwear impressions in criminal investigation[J]. Journal of Liaoning Police College,2011,(01):50-51.

Comparison of DNA Yield and STR DNA Profiles for Different Components of Sperm Differential Extraction Method in Casework Samples

Mrs Gao Linlin[1], Mr Hong Liang[1], Mrs Fu Yanfang[2], Mrs Zhang Mingya[1], Mr Wang Xufeng[1]*
1.Institute of Forensic Science, Hangzhou Public Security Department, China
2.Institute of Forensic Science, Zhejiang Public Security Department, China
The People's Republic of China
**Email: 13738089663@163.com*

Abstract

Successful genotyping of male DNA from mixed samples in sexual assault cases often contributes to the identification of the suspect. Differential lysis is a common method for separating the components of a male-female mixture. However, the method could lose many sperms and result in failed STR genotyping. In this study, we compared the DNA yield and STR DNA profiles from the pellet and the remaining material, and found that large amounts of sperm remained on the original material after the differential cell lysis. In subsequent evaluation of STR genotyping, the profiles of the male component obtained from remaining materials was better than those profiles from the sperm pellets. Therefore, the remaining material in sexual assault cases should not be removed, especially when aged biological evidence material was encountered.

Introduction

Differential lysis to separate male and female components of sexual assault biological samples for autosomal profiling was first described by Gill and colleagues in 1985 [1], and is still the standard method today. In this method, the non-sperm cells are selectively lysed with detergent and proteinase K, while the sperm cells are not lysed due to the highly disulfide cross-linked proteins in the sperm head that resist protease treatment. After the centrifugation, the supernatant containing the victim's DNA is removed, and the pellet containing the sperm is washed several times to remove the remaining victim's DNA before lysing of the sperm with a reducing agent to release soluble and relatively pure male DNA. Unfortunately, the separation process results in the loss of 60-90% of the male DNA [2], although the sperm cell lysis techniques have been improved by many laboratories to increase the male DNA yield and to produce high-quality profiles [3-5]. However, none of these methods tested the remaining biological carrier to analyze whether sperm are still left on the carrier. therefore, this paper aims to propose the necessity of testing the remaining material in sexual assault cases by comparing the DNA recovery yield and STR DNA profiles of the remaining material and the pellet containing the sperm.

Materials and methods

Sample collection and preparation

Thirty-two samples (vaginal swabs, napkins, bed sheets, underwear, etc.) were collected from sexual assault cases. 16 samples were stored at room temperature for less than 12 months which was defined as fresh samples. 16 samples were stored at room temperature for 10 to 25 years which was defined as aged samples (listed in Table 1). A portion of each sample was cut and stored in a 1.5 mL centrifuge tube.

Group of Samples	Number of samples	Cotton swabs	Napkins	Fabric (Underwear, bed sheets, etc.)	Storage time
Fresh samples	16	6	3	7	≤12months
Aged samples	16	8	5	3	10-25years

Table 1. Summary of the samples used for this study.

Cells lysis and DNA extraction

Biological material from each sample was lysed with 1mL ddH_2O, 10μL of proteinase K (20 mg/mL) and 100 μL of 10% SDS followed by incubation at 37°C with shaking at 700 rpm in a Thermomixer comfort (Eppendorf, Germany) overnight. The lysate was transferred to a new tube and centrifuged. The pellet in the new tube and the remaining material left in the original tube were separately washed three times with ddH_2O to remove the residual non-sperm components. DNA from 32 remaining materials and 32 pellets was extracted using the BK-Magnetic beads Based Trace DNA Extraction Kit (24-Channel Automatic Dedicated) on a 24-Channel Automatic DNA Extraction Workstation (BOKUN BIOTECH, China). The procedures were performed according to the manufacturers' instructions with a final elution volume of 40μL.

DNA Quantification

All DNA extracts were quantified using the Quantifiler® Trio DNA Quantification kit (Thermo Fisher Scientific) on the QuantStudio™5 real-time PCR instrument (Thermo Fisher Scientific) following the manufacturer's recommendations, with modifications for half-volume reactions. A total of 5 μL reaction mix, 4 μL primer mix, and 1 μL template DNA were included in per sample. Thermal cycling conditions were as follows: 95°C for 2 minutes, followed by 40 cycles of 95°C for 9 s and 60°C for 30 s. Each DNA extract was repeated to ensure accurate results. DNA yields obtained from the remaining materials and the pellets were compared using a paired t-test (α=0.05).

Amplification and electrophoresis

The VeriFiler™ Plus PCR Amplification kit (Thermo Fisher Scientific, USA) was used to amplify DNA in a Veriti™ 96-Well Thermal Cycler (Life Technologies, USA) according to the manufacturers' instructions. A total of 10 μL of reaction was used for amplification, including 2 μL PCR mix, 1 μL primer mix, 6 μL ddH_2O, and 1 μL of template DNA (≤1 ng total). The reaction was conducted with an initial denaturation at 95℃ for 1 min; 2 cycles of (denaturation at 94℃ for 10 sec, annealing and extension at 62℃ for 90 sec) and 25 cycles of (denaturation at 96℃ for 10 sec, annealing and extension at 59℃ for 90 sec) followed by a final extension step at 60℃ for 5 min. PCR products were prepared for capillary electrophoresis with 1 μL of products, 0.4 μL LIZ600 size standard, and 9 μL Hi-Di™ Formamide per well. Separation and detection of amplified products were performed on a 3500xL Genetic Analyzer (Life Technologies, USA). GeneMapper®ID-X version 1.5 software was used to analyze the collected data. According to the results, 175 relative fluorescence units (RFU) were set as the peak detection threshold for STR allele calling. Peaks less than 15% of the parent peak were removed as a stutter.

Results

DNA Quantification

A significant difference in DNA yields was observed between the remaining materials and the pellets tested ($p = 0.007$, Figure 1). The highest and lowest DNA yields produced from the remaining materials were 52.1968 ng/μL and 0.1154 ng/μL; the DNA yields from pellets were 31.3970 ng/μL and 0.0052 ng/μL, respectively. More importantly, the DNA yield of each remaining material was higher than that of each pellet. Comparing average DNA yields of the remaining materials from fresh-sample group (16.4413±0.5729 ng/μL) to those from aged-sample group (4.6947±0.5951 ng/μL), fresh samples had an increase of approximately 2.5 times in recovered DNA. The average DNA yields with the pellets increased more, with an approximately 5.4 times gain from fresh-sample group (9.1388±0.8440 ng/μL) to those from aged-sample group (1.6987±0.1664 ng/μL). In addition, the ratios of male and female DNA can be evaluated to determine if the separation was successful. In this study, there are 2 mixtures (1:0.6,1:3.8, respectively) of the pellets and 3 mixtures (1:1.5,1:1.2, 1:4.2, respectively) of the remaining materials from fresh samples. All aged samples were the male components.

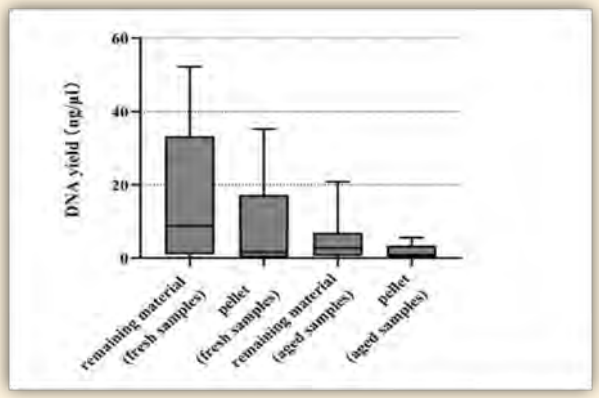


Figure 1. The comparison of mean DNA yields obtained from the remaining materials (n = 32) and the pellets (n = 32) by real -time PCR (qPCR). Significant differences in DNA yields were observed between the remaining materials and the pellets ($p < 0.05$). The mean DNA yield was the highest from the remaining materials of fresh samples and lowest from the pellet of aged samples.

The average quantitation results for all three types of samples were shown in Table 2. Whether fresh samples or aged samples, the difference between the pellets and the remaining materials of the cotton swabs was the lowest.

Group of Samples	Cotton swabs(ng/μL) (pellet/remaining material)	Napkins(ng/μL) (pellet/remaining material)	Fabric(ng/μL) (pellet/remaining material)
Fresh samples	0.2680/3.1150	6.7324/25.0956	17.7737/24.1549
Aged samples	1.3394/3.6689	3.2857/8.0708	0.0118/1.8036

Table 2. Summary of mean DNA yields obtained from the remaining materials and the pellets of three types of samples by real-time PCR (qPCR).

STR Analysis

All STR profiles were analyzed and compared with the goal of determining which were most likely to generate higher quality complete STR profiles. STR profiles of all samples processed in Table 3.

Group of samples	Male STR profile	Mix STR profile	Allele drop-out	No allele
Pellet (fresh samples)	13	2	1	0
Remaining material (fresh samples)	13	3	0	0
Pellet (aged samples)	10	0	5	1
Remaining material (aged samples)	14	0	2	0

Table 3. Comparison of STR profile of the samples used for this study.

Although no significant differences in complete male profiles were observed between the remaining materials and the pellets in the fresh sample group, allele drop-out was detected in D5S818 in one of the pellets. Furthermore, we found that the residual female alleles were detected in some of the pellets from the fresh-sample group. Similarly, the mixed profiles were also observed in the remaining materials of fresh-sample group. None of the profiles from all aged samples were mixed, the remaining materials of aged-sample group generated complete pure male profiles for 14 of the 16 samples (Figure 2), and allele drop-out occurred in fewer large number loci. However, six of the 16 pellets (37.5%) of aged-sample group generated partial or no profiles, demonstrating that DNA yields from the aged samples were lower (Figure 2). As expected, STR success tended to decrease with increasing storage time of the biological materials.

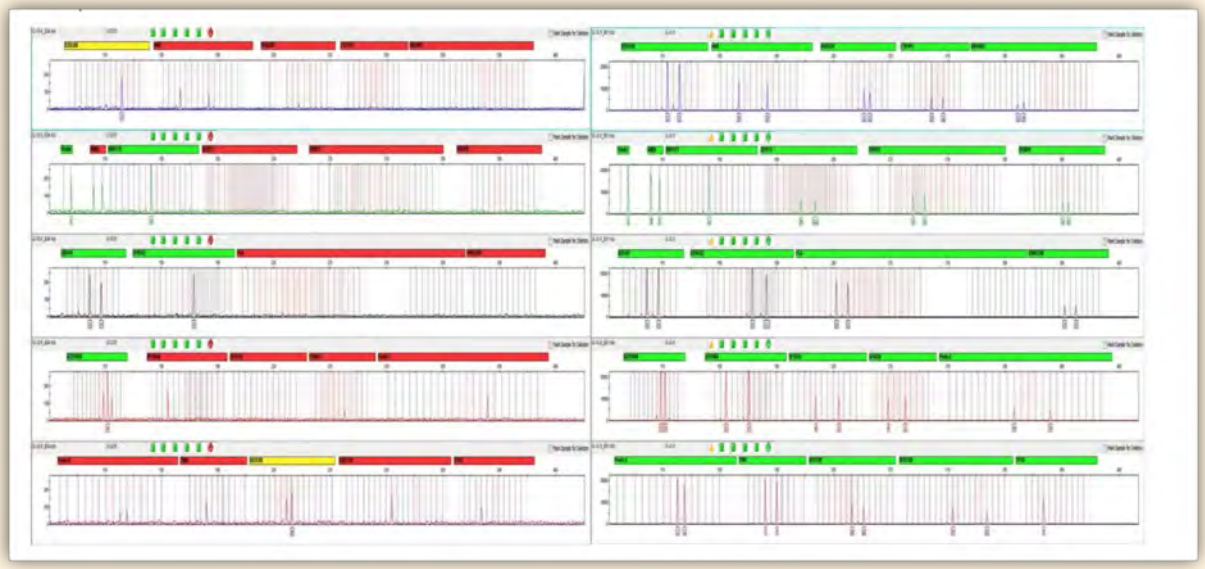


Figure 2. Comparison of STR profile between the pellet (0.0158ng/μL) and the remaining material（1.7908 ng/μL） from the vaginal swab stored at room temperature for 18 years.

Discussion

Differential lysis is typically used for sexual assault evidence to physically separate sperm from epithelial cells. The method takes advantage of differences in both morphology and susceptibility to lysis reagents to enrich for the male contributor and avoid downstream complex mixture profiles. With the development of differential extraction technology, many commercial kits have been used to separate sperm from the suspect and epithelial cells from the victim [4]. To genotype the male DNA, fully automated differential cell lysis/DNA extraction method has been applied in forensic cases [6]. However, the methods mentioned above were mainly for the pellets. In this study, the DNA yields and STR DNA profiles from the remaining materials and the pellets were compared to evaluate sample selection in sexual assault cases.

The differential lysis method recommends at least two washes to remove residual victim DNA. These washing steps have the disadvantage of also removing some sperm, and a significant amount (60–90%) of male DNA can be lost in existing procedures [2]. Furthermore, the swab re-suspension method that led to increased recovery of DNA from cotton swabs demonstrated that much DNA was retained on the swabs after DNA extraction [7]. In the same way, our study shown that only a small fraction of the sperm was suspended in the lysate, and most did not detach from the fibers of the original materials. In the fresh-sample group, the DNA yield of all 7 cloth samples was higher than that of all 6 swab samples, and the amount of DNA recovery of all 5 napkin samples was also higher than that of all 8 swab samples in the old sample group. This result indicates that the release of cells is related to the porosity of the material surface. Cotton swab have a mattress design, cotton is tightly wound around the wooden shaft, it is more ineffective at releasing cellular material. Rocque et al. found that a higher tightness of meshing is disadvantageous releasing DNA profiles for cotton swabs [8], which could also explain DNA yields from cotton swabs was lower than those from other material surfaces.

The complete male STR profile obtained from the remaining material and the failure of the pellet from the same vaginal swab stored at room temperature for 18 years. The size of spermatozoa is much smaller than that of the vaginal epithelial cell, this perhaps results in the spermatozoa more deeply embedded in the fibers. Although shaking the samples during incubation usually yields increased quantities of DNA in the literature [7], it did not been found that the extra step would increase the DNA yields and improve the quality of STR profiles for all aged samples in this study. A previous study has indicated that the affinity between DNA and the fibers on swabs was higher with longer storage time by comparing the DNA recovery efficiency of the same blood dropped onto the same swabs for 1 to 30 days [9]. This may be used to demonstrate the level of DNA recovery for all aged samples decreased seriously. However, the related comparations on the degree of adhesion of DNA to the material surface for longer periods of time, such as in years, needs to be further studied.

Three of the 32 remaining materials were observed mixed profiles, which illustrated the most non-sperm DNA fraction was removed by the differential lysis and multiple washing. The capacities of separating from the biological materials were affected with types of cells. It had been suggested that the spermatozoa do adhere more to the swabs than the other cell types present in semen by comparing the DNA recovery observed for the sperm-loaded samples to that for the azoospermic semen samples [10]. This is thought to be due to the chemical interaction of DNA molecules with the surface functional groups of the materials.

Conclusion

The purpose of this study was to evaluate the need for collection of remaining material in sexual assault cases. Our results showed that the DNA yield from the remaining materials was higher than that from the pellets. This conclusion was supported by obtaining a full STR profile from all remaining materials. Finally, based on the DNA yield and STR genotyping quality obtained in this study, the remaining material in sexual assault cases may become an alternative sample, especially when encountering aged biological evidence material.

References

[1] *Gill P, Jeffreys AJ, Werrett DJ. Forensic application of DNA 'fingerprints'. J. Nature ,1985, 318:577–579.*

[2] *Fatih Inci, Mehmet O. Ozen, Yeseren Saylan, et al. A Novel On-Chip Method for Differential Extraction of Sperm in Forensic Cases.J. ADVANCED SCIENCE,2018, DOI: 10.1002/advs.201800121.*

[3] *Alex M Garvin1, Andrea Fischer, Jutta Schnee-Griese, et al. Isolating DNA from sexual assault cases: a comparison of standard methods with a nuclease-based approach.J. Investigative Genetics, 2012, 3:25, DOI:10.1186/2041-2223-3-25.*

[4] *Schellhammer SK, Hudson BC, Cox JO, et al. Alternative direct-to-amplification sperm cell lysis techniques for sexual assault sample processing.J. Journal of Forensic Science.2022,67:1668–1678.DOI:10.1111/1556-4029.15027.*

[5] *Schwerdtner G, Germann U, Cossu C. The separation of male and female: A comparison of seven protocols. J. Forensic Sci Int Genet Suppl. 2017,6: e9–e11.*

[6] *Matthew C. Goldstein, Jordan O. Cox, Lori B. Seman, et al. Improved resolution of mixed STR profiles using a fully automated differential cell lysis/DNA extraction method.J. FORENSIC SCIENCES RESEARCH, 2020, 5(2), 106–112. DOI:10.1080/20961790.2019.1646479.*

[7] *Michael S. Adamowicz1, Dominique M. Stasulli, Emily M. Sobestanovich, et al. Evaluation of Methods to Improve the Extraction and Recovery of DNA from Cotton Swabs for Forensic Analysis.J. PLOS ONE,2014, DOI:10.1371 / journal.pone. 0116351.*

[8] *Marième J Rocque, Sarah L Leake, Marie-Pierre Milon, et al. The tightness of the cotton swabs meshing influences the chances of getting conclusive DNA Profiles.J. Journal of Forensic Research, 2014, 5(4), DOI:10.4172/2157-7145.1000234.*

[9] *GAO Linlin, ZHOU Zhiquan, LI Youying. Effect of Four Different Swabs on DNA Release from the Adhered-to-swab Human Cells. J. Forensic Science and Technology,2019,44(5),460-462. DOI:10.16467/j.1008-3650-.2019.05.019.*

[10] *Frederic Grosjean, Marylou Favre,Vincent Castella. Comparison between MACSprep™ forensic sperm microbead kit and Erase Sperm Isolation kit for the enrichment of sperm fractions recovered from sexual assault samples.J. International Journal of Legal Medicine, 2023, 137:267–278.*

Determination of DNA Content from Three Types of Bone Sample to Establish the Bone Sampling Guideline for Missing Person and Unidentified Body Examination

Ms Chalampoo Wongworavivat, Ms Nattida Srinak, Ms Somruetai Satmun, Ms Anillada Nettakul, Assistant Professor Dr Worawee Waiyawuth*
Central Institute of Forensic Science, Ministry of Justice, Bangkok, Thailand
**Email: Anillada.n@cifs.mail.go.th, Anil.nettakul@gmail.com*

Abstract

The Central Institute of Forensic Science (CIFS) has been providing DNA testing services to Thai people since 2002. Bone accounts for majority of the biological specimens tested, constituting approximately 26% in total evidence. DNA recovery from the bone is challenging owing to degradation and the presence of inhibitors. Therefore, guidelines for bone selection, extraction, and DNA typing are essential for the routine laboratory of CIFS to maximize DNA yield, and minimize time and cost. In this study, we extracted three types of bones: femur, occipital, and petrous, from 12 bodies using a modified organic extraction and silica-based method. The success rate of the Short Tandem Repeat (STR) typing was determined through the number of reportable loci. Furthermore, analysis of mitochondrial DNA (mtDNA) was performed using the massively parallel sequencing technique. Coverage and variant analyses of all samples were evaluated. The results indicate that the femur exhibits the highest success rate in STR typing. The results, in decreasing order, are as follows: femur > petrous > occipital. We determined that silica-based extraction is the most efficient technique for the STR typing; however, modified organic extraction can be used as an alternative method in obtaining mtDNA. The outcome from this study could serve as a guide for identifying human remains and missing persons in the CIFS laboratory, as well as other Thai forensic laboratories.

Keywords: Missing persons, mitochondrial DNA, massively parallel sequencing

Introduction

The Central Institute of Forensic Science (CIFS) in Thailand was established in 2002 to provide forensic science services to the Thai justice system. The CIFS DNA laboratory processed 1,299 missing person's cases with approximately 5,052 pieces of evidence from 2004-2021. Majority of these biological specimens were bones (1,299 bones), accounting for approximately 26% of the total evidence. Owing to the success of DNA typing, the DNA profiles were imported into the CIFS DNA database.

Critical challenges, such as low DNA content, high levels of degradation, and the presence of potential inhibitors are common in forensic work [1]. DNA accumulation in various parts of bones, including the femur, tooth, and petrous part of the temporal bone [2], has been investigated to identify specimens with high levels of endogenous DNA. Additionally, demineralization techniques and extraction methods have been considered for enhancing the extracted DNA yield. Mitochondrial DNA (mtDNA) identification is used in forensic analyses. Its unique properties, such as its high copy number per cell and ability to determine maternal relationships, make it a valuable tool in human identification [3], especially with the introduction of high-throughput technology. Massively parallel sequencing (MPS) assists in mtDNA sequencing for challenging samples [4].

Operation time and cost are critical considerations for routine laboratory work. Oftentimes, reworking experiments is time-consuming and incurs high costs. Thus, establishing an appropriate protocol to reduce this reworking as much as possible is crucial. Selection of suitable bone parts and the extraction method increases the chances for DNA acquisition from the sample. Utilization of Short Tandem Repeat (STR) typing is reportedly essential evidence in the identification of missing persons, especially in case of sample degradation[1]. Additionally, mtDNA sequencing enhances identification of unidentified bodies and verification of maternal relationship claims [3]. In this study, three types of bone were extracted: femur, occipital and petrous. Two types of extraction methods were used: modified organic extraction and silica-based method. The aim of this study is to evaluate the bone analysis method, including sample selection, extraction, genotyping, and sequencing, to establish a guideline for this process in the CIFS laboratory.

Materials and Methods

Sample selection

Thirty-six human bone fragments from 12 individuals were selected for the analysis. The blood specimens were collected after death. Three years after skeletonization, three different skeletal elements (femur, occipital, and petrous bones) from each individual were examined.

Bone treatment

Potential contamination on the bone sample surfaces was eliminated through washing with 10% sodium hypochlorite, and rinsed with deionized distilled water (ddH2O). Samples were then washed with absolute ethanol, and dried at 56 °C. Finally, the bones were crushed and pulverized in liquid nitrogen using a freezer mill.

Extraction

The bone powder (2 g) was extracted using modified organic extraction method [5]. Another 2 g bone powder was decalcified in 10 mL EDTA (0.5 M, pH 8.0). The QIAamp DNA Blood Maxi kit (Qiagen, Hilden, Germany) was used for silica-based extraction. The protocol was based on Davoren et al.[4], with some modifications.

Quantification and STR genotyping

All samples were quantified using the Quantifiler Trio DNA Quantification Kit (Thermo Fisher Scientific, USA). Autosomal STRs for the bone samples were amplified using the GlobalFiler® PCR Amplification Kit (Thermo Fisher Scientific). The number of reported loci were analyzed using GeneMapper® ID-X version 1.4 follow by the laboratory's criteria.

Figure 1. Images of femur, occipital, and petrous bones. B10F = Body 10 Femur, B10O = Body 10 Occipital, and B10P = Body 10 Petrous bone.

mtDNA sequencing by MPS

Library preparation and template preparation

The mtDNA control region from the bone samples was amplified using the Precision ID Library Kit from the Precision ID mtDNA Control Region Panel (Thermo Fisher Scientific, USA), according to the manufacturer's instructions.

Sequencing and data analysis

Sequencing was performed on an Ion 316™ Chip using the Ion PGM™ Hi-Q™ View Sequencing Kit on an Ion PGM™ System (Thermo Fisher Scientific, USA). Data were analyzed with the Ion Torrent Suite Software (v.5.0.4) using a plug-in variant caller (v.5.0.4.0), coverage analysis (v.5.0.4.0), and HID Genotyper (v.2.1). Variant calls were visualized using Integrative Genomics Viewer (IGV).

Ethical consideration

The research was conducted in accordance with ethical standards for studies involving human subjects. Documentary proof of ethical clearance was obtained from the Research Ethics Review Board at Rangsit University (DPE. no. RSUERB2021-037).

Results

STR typing

DNA profiles of all samples were compared with those of the reference blood samples for reliability. Numbers of the reported loci are presented in Table 1. Notably, the silica-based method provided 677 of the loci, whereas the modified organic extraction method provided 502 of the total 864 loci. The femur and petrous exhibited over 85% and 75% of the loci, respectively, compared to the occipital at below 50%. Femur samples extracted using the silica-based method yielded the most loci (282 loci).

Extraction method	Reported loci (Total 864 loci)	DNA profile type		
		Full	Partial	No profile
Silica-based	677	16	20	0
Modified organic extraction	502	2	34	0
Bone type	**(Total 576 loci)**			
Femur	499	13	11	0
Occipital	232	3	21	0
Petrous	448	2	22	0

Table 1. Number of STR loci by extraction method and by bone type.

mtDNA sequencing

In total, 72 samples were sequenced in six runs. The number of total reads ranged from 846,382 to 1,608,776, with a minimum of 45.70% and maximum of 79.10% aligned reads. The percent read on target ranged from 97.59 - 99.96%, with the exception of samples QB6P and QB6O which were below 40% (30.87% and 39.13%, respectively).

Variants analysis from the Variant Call Format (VCF) report was confirmed manually using the IGV. The number of bases reported was used to determine the profile within the reported ranges (16024-16365, 73-340 and 438-574). The profiles were checked for concordance by comparing them with the reference blood profile. Full mtDNA profile were obtained for 48 of the 72 samples. The remaining 24 samples showed an absence of variance at certain positions. The SB2O and SB4O samples did not yield any variants (silica-based extraction). Modified organic extraction technique resulted 30 full mtDNA profiles whereas the silica-based method resulted 18 profiles. The femur showed the highest number of full mtDNA profile among all the samples (femur 18, occipital 14 and petrous 16 profiles).

Discussion

For STR typing, the silica-based method provided more loci than the modified organic extraction method due to the decalcification process. Inorganic components that interfered with the PCR reactions were removed using a chelating solution[6]. The silica-based method, which takes longer incubation time compared to the modified organic extraction method, results in increased DNA yield and improved acquisition of STR results. According to mtDNA analysis, absence variants were detected in two samples, which is concordant with the STR result. Complete mtDNA profiles were obtained from samples that could not retrieve full STR loci. The mtDNA is located within the mitochondria outside the nucleus and contains a high copy number in each cell, compared to nuclear DNA[3]. This advantage increases the chances of recovering mtDNA from degraded samples [7].

Although STR loci obtained from the petrous and femur were comparable, the femur was selected over the petrous owing to its sampling process. The petrous region is located at the base of the temporal bone, between the sphenoid and occipital regions [8]. To obtain a sample of the petrous part, a craniectomy is required, followed by the use of a small cut-off wheel to delicately cut the bone and avoid damaging other regions that contain osteological information, such as the styloid and mastoid processes. Therefore, appropriate bone types in sample selection are as follows: femur > petrous > occipital. For this study, the best extraction method is the silica-based extraction technique, however, the modified organic extraction can be used as an alternative method. The outcome of this study is essential to the CIFS laboratory for the development of a procedure that can acquire the highest amount of DNA, while also reducing time and cost of repetition.

Conclusion

This study indicates that the best specimen for DNA extraction is the femur, and the results are better in the following decreasing order: femur > petrous > occipital. Among the DNA extraction techniques, silica-based extraction was the most effective technique for STR typing, whereas modified organic extraction is considered an optional method for mtDNA determination. In conclusion, the sample selection, extraction, genotyping, and sequencing methods used in this study will be utilized as guidelines for missing persons and unidentified body examinations in the CIFS laboratory.

Acknowledgements

We would like to thank the entire staff of the Missing and Unidentified Persons System Development Division and the Division of Forensic DNA, CIFS for their technical assistance. We acknowledge the assistance provided by Narumol Parasompong for guidance on sample selection.

References

[1] Loreille OM, Diegoli TM, Irwin JA, Coble MD, Parsons TJ. High efficiency DNA extraction from bone by total demineralization. Forensic Science International: Genetics. 2007;1(2):191–5. https://doi.org/10.1016/j .fsigen.2007.02.006.

[2] Miloš A, Selmanović A, Smajlović L, Huel RL, Katzmarzyk C, Rizvić A, Parsons TJ. Success rates of nuclear short tandem repeat typing from different skeletal elements. Croatian medical journal. 2007 Aug;48(4):486.

[3] Melton T, Holland C, Holland M. Forensic mitochondria DNA analysis: current practice and future potential. Forensic science review. 2012 Jul 1;24(2):101.

[4] Davoren J, Vanek D, Konjhodzić R, Crews J, Huffine E, Parsons TJ. Highly effective DNA extraction method for nuclear short tandem repeat testing of skeletal remains from mass graves. Croatian medical journal. 2007 Aug;48(4):478.

[5] Ambers A, Gill-King H, Dirkmaat D, Benjamin R, King J, Budowle B. Autosomal and Y-STR analysis of degraded DNA from the 120-year-old skeletal remains of Ezekiel Harper. Forensic Science International: Genetics. 2014 Mar 1; 9:33-41.

[6] Mckinnon M, Henneberg M, Higgins D. A review of the current understanding of burned bone as a source of DNA for human identification. Science & Justice. 2021 Jul 1;61(4):332-8. https://doi.org/10.1016/j.scijus.2021.03.006.

[7] Latham KE, Miller JJ. DNA recovery and analysis from skeletal material in modern forensic contexts. Forensic sciences research. 2019 Mar;4(1):51-9. https://doi.org/10.1080/20961790.2018.1515594

[8] Charlton S, Booth T, Barnes I. The problem with petrous? A consideration of the potential biases in the utilization of pars petrosa for ancient DNA analysis. World Archaeology. 2019 Aug 8;51(4):574-85. https:// doi.org/10.1080/00438243.2019.1694062

Evaluation Study of RSID™- Semen with Universal Buffer in Comparison with SERATEC® PSA Semiquant for Rapid Forensic Identification of Semen: Preliminary Results

Erizasyira Binti Basri, Allia Binti Shahril, Nurul Amira Binti Mohamed Farid, Siti Hajar Binti Hussin, Nor Aidora Binti Saedon*
Forensic DNA Division, Department of Chemistry, Malaysia
**Email: erizasyira@kimia.gov.my*

Abstract

Identification of biological fluid through screening and confirmatory test is an important aspect in allowing subsequent analysis by DNA profiling. A reliable and rapid confirmatory method is needed to determine the presence of semen from sexual assault evidence. The aim of this study is to perform a preliminary evaluation of the RSID™-Semen in detecting semenogelin (Sg) in comparison with the SERATEC® PSA Semiquant in detecting prostate specific antigen (PSA) in semen which currently in use by incorporating several parameters: sensitivity, specificity and two bodily fluid mixtures. In conclusion, both methods exhibit high sensitivity to PSA and Sg beyond 1:32 dilutions. Based on a 1:1 ratio of semen to other bodily fluids, SERATEC® PSA Semiquant and RSID™-Semen are both capable of detecting semen in mixtures. Compared to SERATEC® PSA Semiquant, the RSID™-Semen with Universal Buffer showed greater specificity for the identification of semen.

Introduction

Biological evidence found at the crime scene can help investigators determine the nature of the crime and provide leads. Cases of sexual assault are frequently thought of as hidden crimes where the only witnesses are the victim and the assailant hence semen stain is the only crucial witness. Presumptive tests are typically conducted to rule out or include the evidence for subsequent tests [1]. The used of alternate light source (ALS) for screening at the crime scene is however not specific because other biological stains may also fluoresce when exposed to the light [2]. Acid-Phosphatase (AP) testing, which detects enzyme secreted by the prostate gland, is another presumptive method for detection of semen fluid [3]. However, the presence of common household items like plant matter, feminine hygiene products, and vaginal secretions can cause an AP test to result in a false positive [4].

Microscopical examination of spermatozoa has been used as a confirmatory test for semen stain identification, but this can be hampered by the presence of other cells in the samples, the malformation or degradation of spermatozoa, or low sperm counts or azoospermia due to vasectomy or other medical conditions [4]. Acid phosphatase, an enzyme secreted by the prostate gland, is found in high concentrations in human semen and is detected using PSA test strip. This immunochromatographic assay represents a more precise marker, but it can also produce false-positive results for urine, vaginal fluids, breast milk, and rectal swabs, albeit in small amounts [5]. Additionally, it has been demonstrated that PSA exhibits variable stabilities in vaginal fluids, which could lead to incorrect interpretation of the results [4].

RSID™-Semen test strip is an immunochromatographic test strip designed to detect presence of human semenogelin (Sg). Human seminal plasma contains a significant amount of the protein semenogelin, which is produced in seminal vesicles. Sg is present in small amounts in several other tissues, including those of the trachea, kidney, colon, and lung, but these tissues are not encountered in sexual assault cases. An advantage over PSA testing is that no Sg is found in the female genital cavity which eliminate the possibility of false positive [4,6].

The objective of this study is to perform a preliminary study to compare the effectiveness of the RSID™-Semen for semen identification in comparison with the currently employed method SERATEC® PSA Semiquant under various parameters; sensitivity, specificity, and the capacity to identify semen when it is mixed with other biological fluids (blood, saliva, urine, and breast milk). The semen sample for sensitivity and body fluid mixtures study was obtained from one male donor which was used by the laboratory as quality control (QC) sample.

Material and Methods

Sample preparation

A male volunteer's seminal fluid was obtained, and the DNA of the semen was extracted using Chelex method and quantified using Real-Time PCR to determine the estimated concentration of DNA in the seminal fluid. The estimated amount of DNA in 1 µL of semen fluid is 2.8903ng/µL.

Study Parameters

The study encompassed several parameters as presented in Table 1.

Parameter	Preparation
Sensitivity	Two-fold serial dilution of up to six dilutions were carried out and each were separately deposited on a cotton swab.
Specificity	Urine (from three males and two female donors) / saliva (from two males and one female donors) / breast milk (from two female donors); 50 µL of the body fluid from each individual were deposited on a sterile cotton swab separately.
Body fluid mixture (1:1 ratio)	Semen: Blood (S:B) / Semen:Breast milk (S:BM) / Semen:Saliva (S:SL) / Semen:Urine (S:U); 50 µL of body fluid were deposited on sterile cotton swab and cloth.

Table 1. The parameters used and preparation of samples. All samples were prepared in duplicate for use in the RFID™-Semen and SERATEC® PSA Semiquant.

Test kit procedures

SERATEC® PSA Semiquant

Approximately a 1-cm^2 semen stain or half a cotton swab was cut and placed into a 1.5 mL microfuge tube. The sample was extracted in 1 mL of extraction buffer (10 mM Tris, 10 mM disodium EDTA, 10 mM sodium chloride) on a shaker thermomixer for 1 hour at 38 ºC. About 200 µL of the extracted sample was load into the sample well. The result was evaluated after 10 minutes of sample addition. The presence of a line between the control (C) and test (T) lines indicates a positive result; the absence of a line at the T line denotes a negative result.

RSID™-Semen with Universal Buffer

Approximately a 1-cm^2 semen stain or half a cotton swab was cut and placed into a 1.5 mL microfuge tube. The sample was extracted in 300 µL of RSID™-Universal Buffer in shaker thermomixer for 1 hour at 38 ºC. About 20 µL of the extracted sample was diluted in 80 µL of RSID™-Universal Buffer (total volume 100 µL) and was load into sample well. The result was evaluated after 10 minutes of sample addition. A visible red line in both the control (C) and test (T) lines of the RSID test strip indicates a positive result; the absence of a line at the T line denotes a negative result.

Quality control for RSID™-Semen with Universal Buffer

The positive control was prepared by depositing 50 µL of human semen on cotton swab. It was extracted in 1 mL of RSID™-Universal Buffer for 1 hour at 38 ºC in shaker thermomixer and 5 µL of this extract was diluted in 95 µL of RSID™-Universal Buffer (total volume 100 µL RSID™-Universal Buffer) whilst for negative control, a sterile cotton swab was extracted in 300 µL RSID™-Universal Buffer and 100 µL of each control was load into the sample well.

Results and Discussion

Sensitivity

RSID™-Semen and SERATEC® PSA Semiquant both demonstrate their sensitivity to different semen concentrations resulting from a series of dilutions as shown in Figure 1. SERATEC® PSA Semiquant detected signals at a higher intensity since it used undiluted sample whereas RSID™-Semen's samples were diluted prior loading into the sample well to avoid high-dose hook effect. Both RSID™-Semen and SERATEC® PSA Semiquant can detect semen in minute amount beyond 1:32 dilutions. The RSID™-Semen is a confirmatory qualitative test that can detect as little as 2.5 nL of human semen [7]. Based on Laffan et al., (2019), The SERATEC® PSA Semiquant has a limit of detection of 200 ng/mL as compared to 8.0 x 10(3) ng/mL, making it more sensitive than the RSID-Semen kit [6].

Figure 1. Sensitivity of RSID™-Semen and SERATEC® PSA Semiquant of semen extract.

Specificity

The urine extracts tested with RSID™-Semen detected one positive result from a male individual (M2) as shown in Figure 2 while the remaining of the samples were tested negative. This positive result can be explained by the possibility of traces amount of semen presence in urine sample taken since it came from the same passage. Conversely, positive results from a female (F1) and three male individuals (M1, M2, and M3) were discovered when tested for PSA. None of the saliva or breast milk extracts were tested positive for Sg and PSA. PSA is present in urine and vaginal fluids, and it can cause a false-positive in the PSA test strip for vaginal swabs [7]. As demonstrated in the current study, PSA was found in urine sample from both men and woman which have the potential to test falsely positive on PSA test strips, albeit in very small amounts [8]. According to Laffan et al., (2011), in contrast to the 2.9% false-negatives with the SERATEC® PSA Semiquant, the RSID™-Semen produced no false results. The RSID™-Semen, according to Pang and Cheung et al., (2007) is more sensitive to detecting Sg in semen samples extracted from vaginal fluid [9]. According to the current study, RSID™-Semen demonstrates higher advantage to SERATEC® PSA Semiquant.

Figure 2. Specificity of RSID™-Semen and SERATEC® PSA Semiquant of urine extract.

Body Fluid Mixtures

The detection of semen in both RSID™-Semen and SERATEC® PSA Semiquant is unaffected by the mixtures of semen: blood, semen: urine, semen: saliva, and semen: breast milk. The substrates used in this study, cotton swabs and cloth, both offer high semen fluid absorbencies and as a result, have no impact on the recovery of PSA or Sg in SERATEC® PSA Semiquant and RSID™-Semen test strip. PSA or Sg in semen can be detected even when it mixed with other biological fluids. However, PSA cannot be effectively detected 24 to 48 hours after sexual activity because of the quick decay of PSA in vaginal fluid over time [10].

Quality Control for RSID™-SEMEN with Universal Buffer

The positive control gave a visible red line at the control (C) and test (T) that indicates a positive result whilst negative control sample gave a visible red line at the control (C) position only that indicates a negative result.

Conclusion

Compared to SERATEC® PSA Semiquant, the RSID™-Semen with Universal Buffer showed greater specificity for the identification of semen. Both methods exhibit high sensitivity to PSA and Sg respectively beyond 1:32 dilutions and based on a 1:1 ratio of semen to other bodily fluids, both are capable of detecting semen in mixtures.

References

[1] *Lewis J, Baird A, McAlister C, Siemieniuk A, Blackmore L, McCabe B, O'Rourke P, Parekh R, Watson E, Wheelhouse M, Wilson N. Improved detection of semen by use of direct acid phosphatase testing. Sci Justice. 2013 Dec;53(4):385-94. doi: 10.1016/j.scijus.2013.04.009. Epub 2013 Jun 26. PMID: 24188339.*

[2] *Nelson DG, Santucci KA. An alternate light source to detect semen. Acad Emerg Med. 2002 Oct;9(10):1045-8. doi: 10.1111/j.1553-2712.2002.tb02139.x. PMID: 12359543.*

[3] *Sijen T, Harbison S. On the Identification of Body Fluids and Tissues: A Crucial Link in the Investigation and Solution of Crime. Genes (Basel). 2021 Oct 28;12(11):1728. doi: 10.3390/genes12111728. PMID: 34828334; PMCID: PMC8617621.*

[4] *Old, J., Schweers, B.A., Boonlayangoor, P.W., Fischer, B., Miller, K.W.P. and Reich, K. (2012), Developmental Validation of RSID™-Semen: A Lateral Flow Immunochromatographic Strip Test for the Forensic Detection of Human Semen. Journal of Forensic Sciences, 57: 489-499. https://doi.org/10.1111/j.1556-4029.2011.01968.x*

[5] *Bitner SE. False positives observed on the Seratec® PSA Semiquant Cassette Test with condom lubricants. J Forensic Sci. 2012 Nov;57(6):1545-8. doi: 10.1111/j.1556-4029.2012.02141.x. Epub 2012 Apr 11. PMID: 22494324.*

[6] *Laffan A, Sawyer I, Quinones I, Daniel B. Evaluation of semen presumptive tests for use at crime scenes. Med Sci Law. 2011 Jan;51(1):11-7. doi: 10.1258/msl.2010.010040. PMID: 21595415.*

[7] *Martínez P, Santiago B, Alcalá B, Atienza I. Semen searching when sperm is absent. Sci Justice. 2015 Mar;55(2):118-23. doi: 10.1016/j.scijus.2015.01.008. Epub 2015 Feb 17. PMID: 25753997.*

[8] *Harbison S, Fleming R. Forensic body fluid identification: state of the art. Research and Reports in Forensic Medical Science 2016;6:11-23 https://doi.org/10.2147/RRFMS.S57994.*

[9] *Pang BC, Cheung BK. Identification of human semenogelin in membrane strip test as an alternative method for the detection of semen. Forensic Sci Int. 2007 Jun 14;169(1):27-31. doi: 10.1016/j.forsciint.2006.07.021. Epub 2006 Sep 1. PMID: 16949235.*

[10] *Culhane JF, Nyirjesy P, McCollum K, Casabellata G, Di Santolo M, Cauci S. Evaluation of semen detection in vaginal secretions: comparison of four methods. Am J Reprod Immunol. 2008 Sep;60(3):274-81. doi: 10.1111/j.1600-0897.2008.00632.x. Epub 2008 Jul 18. PMID: 18647289.*

Assessment of Cross-Contamination Risks Associated with the Use of Unlined Metal Cans with Fire Debris Containing Petrol in Oven Heating

Mrs Nawgalage Induma Kalpani Fernando, Ms M. M. D. Jayarathna, W. Alex Rushan Fernando, Prof S. D. A. Sandanayaka.*

Government Analyst's Department, Sri Lanka

**Email: indu.fernandogad@gmail.com*

Abstract

Like many other countries, Sri Lanka also widely uses metal cans due to their low costs in addition to nylon bags to collect fire debris samples. However, the use of lined cans can introduce extraneous interfering compounds, due to the co-extraction of volatiles from the can's lining, which may lead to false data interpretation [1]. To avoid this problem, unlined metal cans are recommended. Nevertheless, the use of unlined cans has another challenge since they are not completely airtight. Then, volatile compounds can escape and re-enter other unlined metal cans when storing, transporting, or oven heating. The presented study aimed to investigate this cross-contamination behaviour of unlined metal cans with fire debris samples containing different amounts of petrol subjected to oven heating. Sample concentration was done according to the ASTM E-1412 and analysis was carried out by GC/MS. Data interpretation was done as described in ASTM E-1618 [2, 3].

Cross-contamination behaviour was found to be quite significant and had a relationship with the amount of petrol spiked.

Introduction

Fire is an awesome and smart discovery of the means of making human lifestyles more comfortable ever since the Old Stone Age. The pathetic situation is that eventually due to the destructive and uncontrollable nature of fire, many disasters occur by killing people, destroying properties in the world. As a result, the necessity of conducting a proper arson investigation has arisen to determine the cause and origin of the fires.

Evidence from a fire scene could contain ignitable liquid residues (ILR) that could be useful in an arson investigation. By continuing investigations along this path, depending on the laws, criminals are prosecuted if the cause of the fire was found to be committed intentionally. Practically, the amount of ILR contained in a fire debris sample varies from one location to another. So, it may contain a trace amount or comparatively large amount, or even undetectable amounts of used accelerant. Due to the volatile nature of accelerants, this can cause cross-contamination across samples even before they are heated in an oven. Therefore, care should be made to handle the samples correctly throughout transport and storage before sending them for laboratory analysis. Any form of package is susceptible to contamination and cross-contamination [4, 5].

In this research, unlined metal cans were tested to study their airtightness when the sample cans were subjected to oven heating in passive headspace extraction. By preparing spiked samples of fresh petrol ranging from 1µL to 50µL of petrol, the cans' airtightness was studied. Petrol was chosen because most of the fires are accelerated with petrol since it is readily available and inexpensive.

Volatile compounds were solvent-extracted using heated passive headspace adsorption by charcoal strips followed by acetone. Analyses were performed using gas chromatography/mass spectrometry (GC/MS), and data interpretation was conducted by visually comparing the total ion chromatograms (TIC) with reference chromatograms. Additional data analysis was performed using extracted ion profiling (EIP), target compound analysis, or both as given in the ASTM E-1618.

In this work, cross-contamination occurred with a recognized pattern distortion associated with the less spiked volumes and without any pattern distortion associated with the higher spiked volumes in their chromatograms. This is due to the different leakage rates between more volatile components though less abundant and slightly less volatile components though more abundant in petrol. It is crucial to note that even minor contamination of fire debris evidence may produce misleading results in forensic cases of arson. That is why experts must know to interpret the pattern distortions as well as the necessity of analysing control samples collected along with the samples of fire debris to minimize cross-contamination problems.

Research Methodology

Chemicals and Instrumentation

Samples of wood shavings, acetone (99%), HPLC grade (Sisco Research Laboratories Pvt. Ltd), Unlined metal cans of 1 L (Mikro Industries, Sri Lanka), nylon bags of (12''x18'') from Arrowhead Forensics, USA, activated charcoal strips (20mm×8mm×1mm) from Arrowhead Forensics, USA.

Forced convection oven (DK-600DT, Yihder Technologies Co., Ltd, Taiwan), GC/MS (Agilent Technologies 7890B GC, 5977A MSD, 7693 Autosampler).

Method

Two samples of wood shavings (10 g) were placed into two metal canes (1 L) separately. While keeping one of the cans as it is, a particular volume of petrol is added to the other can (1 µL, 3 µL, 5 µL, 10 µL 15 µL, 30 µL, and 50 µL). Both cans were kept in a nylon bag. An activated charcoal strip was suspended inside both the petrol-free can and the outer nylon bag to identify escaped volatile compounds from the spike cans (Figure: 1). Each experiment was done in duplicate, and they were subjected to oven heating at 80^0C for 8 hours for passive headspace extraction. The analyses were carried out using GC/MS using a temperature program as given in Table 1. Resultant chromatograms were used for the data analysis, mainly by visual comparison with the reference petrol chromatogram. Additional data analysis was carried out using EIP, target compound analysis, or both. NIST external library was used for target compound identifications.

Figure 1. Schematic representation of the experimental setup for cross-contamination tests. A full-size nylon bag (12"x18") containing two 1L volume unlined metal cans with activated charcoal strips.

Initial temperature	40^0C
Initial hold time	2 min
1st Ramp rate	5^0C/min up to 90^0C
2nd Ramp rate	14^0C/ min up to 250^0C
Hold time	10 min
Final temperature	250^0 C
Total run time	33.5 min

Table 1. GC temperature program.

Results and findings

It is not necessary to have a complete pattern of target compounds of petrol to identify contamination in the cross-contamination experiments. Cross-contamination should be considered if a partial pattern or the presence of some compounds from the reference petrol was detected as it may generate a false-positive result. When a compound found in petrol was identified in an adjacent, petrol free unlined metal can, the results were declared positive for cross-contamination in this investigation.

Chromatograms for both the control nylon bag and the control unlined can did not show any peaks in addition to peaks in the solvent blank. Peaks obtained for the chromatogram of wood shavings also did not interfere with the target petrol compounds, as shown in the Figure 2.

The EIPs obtained for neighbouring un-spiked cans associated with each test volume compared with the reference petrol are shown in Figure 3.

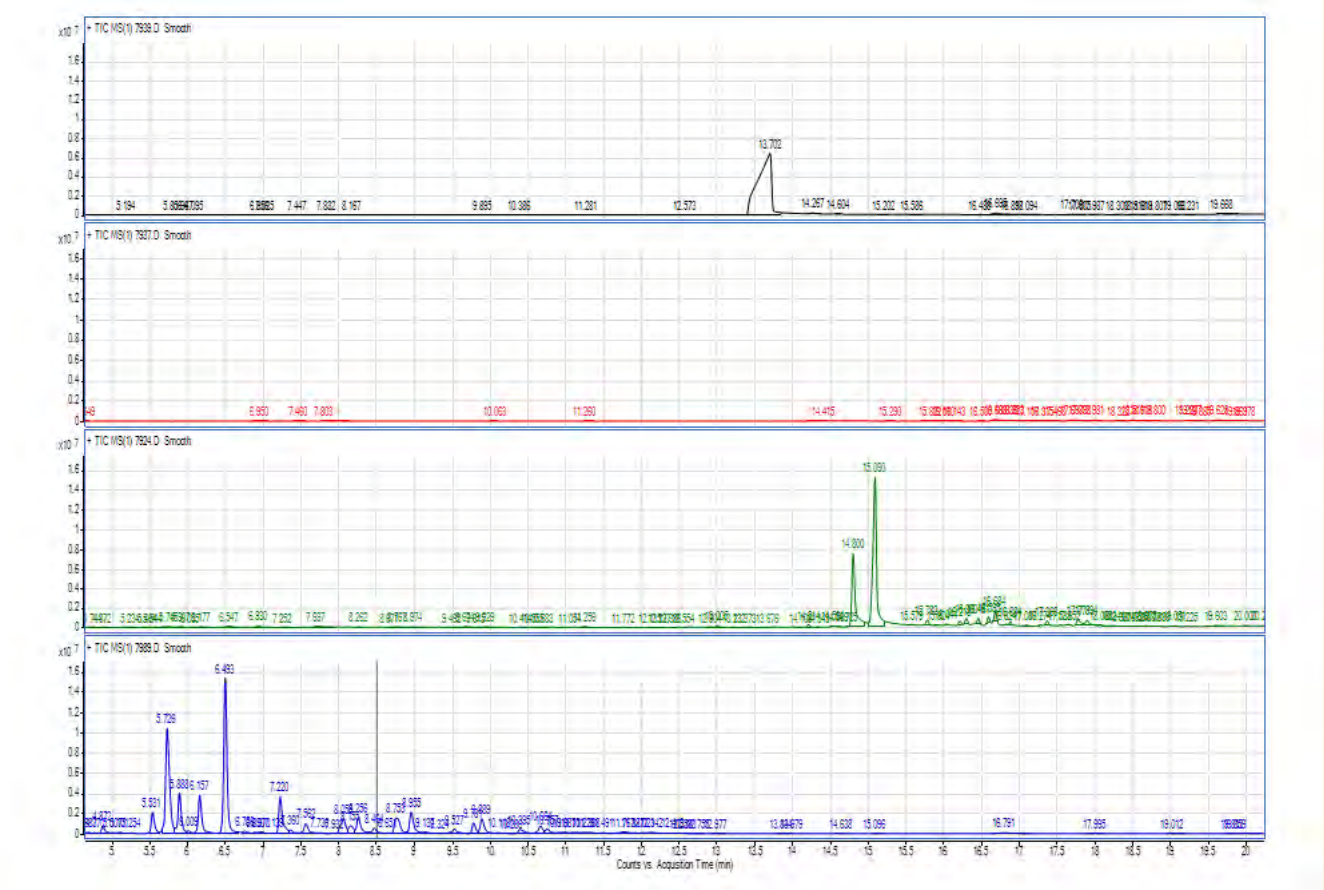


Figure 2. From the top: TIC for control nylon bag, control unlined metal can and control sample of wood shavings and (D)0.1% petrol reference.

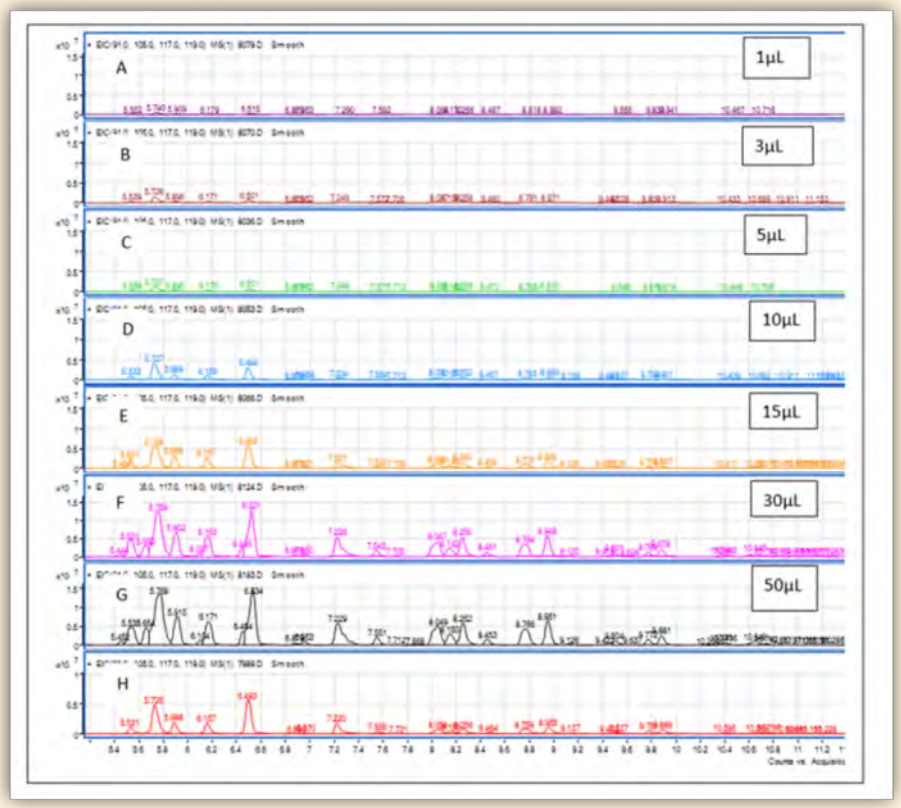


Figure 3. EIPs of un-spiked metal cans tested for cross-contamination for the known volumes from the top; (A)1µL, (B)3µL, (C)5µL, (D)10µL, (E)15µL, (F)30µL, (G)50µL, (H)0.1% gasoline reference.

In forensic fire investigation, ILR identification is a qualitative aspect typically based on accelerant pattern recognition visually. When a sample is subjected to cross-contamination, a distortion of the diagnostic pattern could be seen. 1µL was the minimum volume of fresh petrol measured for the study, and this amount was already significant enough to contribute to the contamination of the neighbouring petrol-free metal can, as indicated in the chromatogram (A) in Figure 3. The charcoal strips placed outside the metal tins were originally intended to be analysed to determine if petrol had leaked out from the cans. However, contamination could be observed from all the cans and therefore, the charcoal strips outside the metal cans were eventually not required to be analysed. The highly volatile light molecules until indane have made their way to the nearby metal can causing cross-contamination. Therefore, the characteristic petrol pattern has deviated when it comes to the cross-contamination of petrol from unlined metal cans. In a reference petrol sample, the most abundant aromatic peak is 1,2,4-trimethylbenzene whereas the second most abundant peak is the co-eluting peak of 1-ethyl-3-methylbenzene / 1-ethyl-4-methylbenzene. Despite the fact that 1,2,4-trimethylbenzene is the prominent peak in reference petrol, the highest abundant peak in a cross-contaminated sample of 1µL petrol was the co-eluting peak of 1-ethyl-3-methylbenzene / 1-ethyl-4-methylbenzene. It is clear that the highly volatile, lighter compounds that appeared at the early stage of the chromatogram have a greater tendency to cross-talk with adjacent samples than less volatile, slightly heavier compounds when cross-contaminated with low quantities of petrol. The co-eluting peak is the second most abundant peak in the reference petrol and these lighter molecules can leak first and re-enter through the joints of the can's surface due to their high volatility, even though they are present in slightly lower abundances.

The pattern for 3µL which is illustrated in (B) Figure 3, was also distinct from the neat petrol detection pattern and comparable to the pattern obtained for 1µL. When increasing the added petrol volume, the 1,2,4-trimethylbenzene peak competes to overcome the co-eluting peak of 1-ethyl-3-methylbenzene / 1-ethyl-4-methylbenzene. The height difference between the two peaks gradually reduces as the volume increases up to 10µL. When considering these peaks' heights difference in 15µL, it is too small and has started to transform the peak pattern as in reference petrol. That means 1,2,4-trimethylbenzene appeared as the more prominent peak in the chromatogram. Thereafter, these two peaks resemble those in reference petrol for further increased volumes of petrol up to 50µL as shown in Figure 4. Therefore, no significant difference in peak pattern in the chromatogram, especially the two abundant peaks compared to the reference petrol means cross-contamination occurs at significant quantities of petrol. The reason for this behaviour is the leaking of highly volatile co-eluting components (second most abundant components) , as well as more abundant component slightly less volatile, are competing to escape from metal cans and re-enter nearby cans at a similar rate, resulting in the same diagnostic peak pattern as in the reference petrol in the chromatogram.

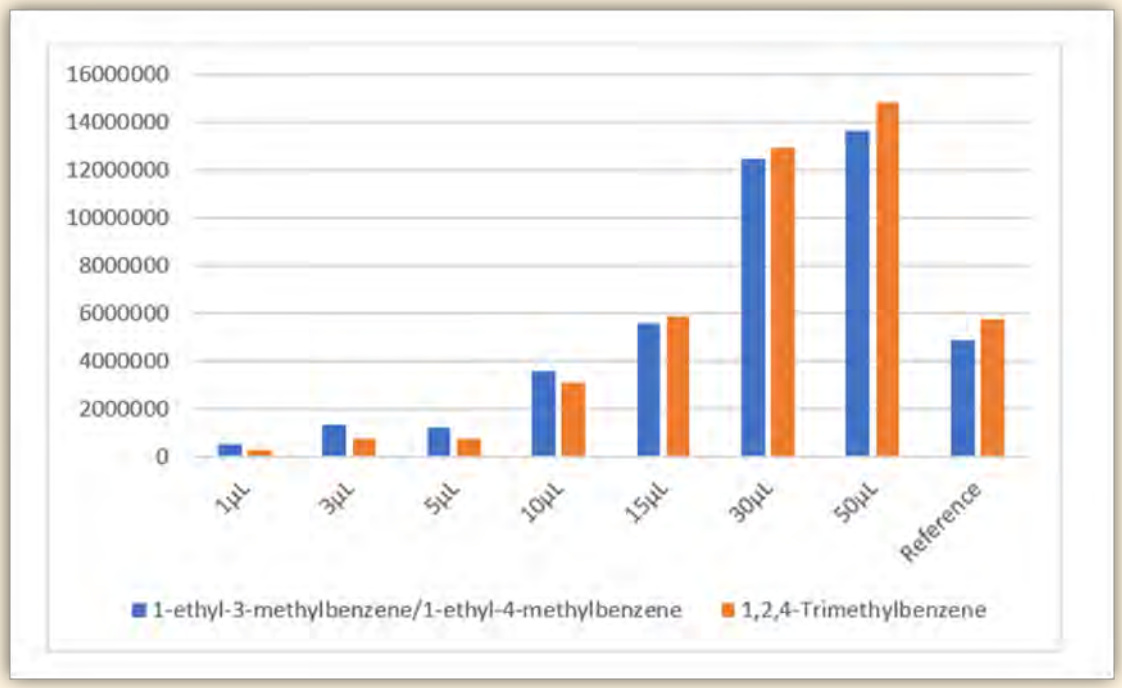


Figure 4. Comparison of two peak heights (1-ethyl-3-methylbenzene and 1,2,4-Trimethylbenzene) of 1µL, 3µL, 5µL, 10µL, 15µL, 30µL, 50µL and 0.1% gasoline reference.

Conclusions, implications, and significance

To make conclusion as positive for petrol, specific aromatic compounds are considered by the laboratory. Those include castle group, 1,2,4-trimethylbenzene, 1,2,3-trimethylbenzene and additionally indane and C4 benzene. Hence, this means that for partial profile detected in the contaminated samples, petrol would not be reported.

The study revealed that cross-contamination can cause pattern distortion in the chromatograms, with the co-eluting peak of 1-ethyl-3-methylbenzene / 1-ethyl-4-methylbenzene appearing as the tallest peak in low levels of spiked petrol samples. In high levels of spiked petrol samples, both the co-eluting peak of the light end of the chromatogram and the 1,2,4-trimethylbenzene peak resemble those in reference petrol without any pattern distortion. However, in both cases, it is more likely to be interpreted results as false positives for petrol since all the target compounds were detected.

In addition to cross-contamination, background interference coming from the fire site while materials are burning also causes pattern distortions. To resolve this disagreement, the fire investigators are recommended to collect comparison samples to subtract interferences generated by burnt and pyrolyzed products that could produce aromatics. To avoid cross-contamination issues, the laboratory analyst should analyse control samples (empty cans) produced at the fire scene thoroughly in addition to debris samples. Further, when samples of fire debris are analysed, peak patterns with relative abundance should be considered before a conclusion is drawn.

It is suggested that fire debris samples with heavy-loaded accelerants collected in unlined metal cans be kept, transported, stored, and heated separately, or in circumstances where this is not possible, nylon bags should be used instead for sampling fire debris. Double bagging is a common practice when nylon bags are used for packaging heavy-loaded fire debris samples [4].

References

[1] *Stauffer E, Dolan JA, Newman R. Fire Debris Analysis. Academic Press; 2008.*

[2] *ASTM International. ASTM E1412-16 Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration with Activated Charcoal [Internet]. West Conshohocken (PA): ASTM International; 2016 [cited 2023 Apr 12]. Available from: https://www.astm.org/DATABASE.CART/HISTORICAL/E1412-16.htm*

[3] *ASTM International. ASTM E1618-14 Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry [Internet]. West Conshohocken (PA): ASTM International; 2014 [cited 2023 Apr 12]. Available from: https://www.astm.org/DATABASE.CART/HISTORICAL/E1618-14.htm*

[4] *Belchior F, Andrews SP. Evaluation of Cross contamination of Nylon Bags with Heavy-loaded Gasoline Fire Debris and with Automotive Paint Thinner. Journal of forensic sciences. 2016 Nov;61(6):1622-31.*

[5] *Williams MR, Sigman M. Performance testing of commercial containers for collection and storage of fire debris evidence. Journal of forensic sciences. 2007 May;52(3):579-85.*

The Impacts of Skills, Attitude, Time Management, Technical Equipment And Work Experience on Work Performance: The Case of National Forensic Agency of Mongolia

*Mr LKHAGVASUREN Batkhishig [1] Mrs TUMENNAST Munkhbaatar [2]**
[1] Ph.D, Director of Forensic Science Institute at University of Internal Affairs, Mongolia, Police Lieutenant Colonel
[2] Ph.D, Head of Education, Scientific Research and Development Center, National Forensic Agency of Mongolia, Police Major
National Forensic Agency of Mongolia, Mongolia
**Email: Tumennast.0413@gmail.com*

Abstract

This study aims to investigate if the five factors (professional skills, attitude, time management, technical equipment, and experience) have a direct relationship with the work performed on the work performance of a forensic signature expert at the National Forensic Agency of Mongolia. In other countries, many scholars studied the related factors of performance management in the world, but there is a lack of study on our topic in the Mongolian forensic science sector. Thus, we are interested in this topic.

Performance management involves the process of all activities which occurs between an employer and an employee as support of accomplishing value, mission, vision and main objectives in the organization.

The data was collected from 46 experts who work in the National Forensic Agency of Mongolia. The result of data determined online in the first quarter of 2022 and estimated by SMART PLS 3.0 software and Cronbach's alpha index have been used for data analysis and reliability analysis of the questionnaire, respectively in our study. In our study from many others we analyzed five hypotheses, one of them had a positive relationship with considered impacts. On the other hand, the four hypotheses could not have a positive relationship on considered impacts. Research has established the significant impact such as experience on work performance in this study.

Keywords: University of Internal Affairs, Mongolia (UIAM), professional skills, time management, attitudes, technique equipment, experience, work performance

Introduction

The work performance of the organization is crucial. The super-objective of all organizations is to improve their performance. The definition of work performance varies from country to country, but there are still challenges regarding objectivity and fairness. To determine work performance, each employee should evaluate how well he or she performs his or her duties, what results are being achieved, and his or her skills, qualifications, and workload. This will provide the organization with real-time information about the performance of the work, as well as reward the employee, provide training or correction, and provide the necessary equipment. The main objective of our study is to measure the impacts of professional skills, attitude, time management, technical equipment, and experience on the work performance of a forensic signature expert at the National Forensic Agency of Mongolia.

Conceptual Framework

It means that the quality and results of the expert conclusion will be improved by developing the methodology of the signature examination.

Therefore, based on the basic theory of work performance and the research results of international researchers, we identified the factors that directly affect the work performance of forensic signature experts working in the Mongolian Forensic Agency. Furthermore, we have determined the quality, hypothesis and model of the research work.

The survey was conducted electronically by 46 officers who were and are performing signature analysis, and using the internationally recognized qualitative research program SMART PLS-3.0, quantitative, correlational, multivariate, and path analysis was performed to determine the variables of the factor within the signature analysis experts. conducted a study to prove how it affects work performance and its relevance, and summarized the results (Figure 2).

Although the issue of determining job performance varies from country to country, it still poses some problems in terms of objectivity and fairness. In order to determine work performance, it is necessary to evaluate how each employee fulfils the tasks assigned to him and what results are achieved, taking into account his skills and professional training and workload. In this way, the organization gets correctness information about the performance of the work, and further, it is crucial to take measures such as rewarding the employee, training him or correcting the

Professional skills and work performance

There many scholars studied assume that a link between skill and performance. Irena Grugulis, and Dimitrinka Stoyanova (Grugulis, 2011), highlighted the problems involved in capturing, measuring, and linking skill and performance. Indeed, much of the activity and interest in this area is predicated on the existence of such a link and the likelihood, in the words of the title of the Leitch Report (Claire Johnson, 2006), that prosperity for all will come from world-class skills. Such a link does exist. Individuals can add to their lifetime earnings, decrease the likelihood and length of unemployment, and secure more readable work by obtaining particular qualifications (Stephen Machin, 2001), while professional bodies and trade unions can gain higher wages and greater levels of influence for their members (Rhoades, 1983). Training has been linked to higher profits in firms "Hambledon Group Ltd." and skill differentials form an enduring aspect of national differences in productivity (Stoyanova, 2011). According to the literature review, we hypothesized as below:

H1. Professional skills positive related on work performance.

- *Good decision-making skills*
- *Use of forensic examination methods*
- *Observantness and prudence*
- *Thinking skills*
- *Skills which write examination conclusions*

Furthermore, we considered the following parameters when examining work performance concerning other factors. It includes the appreciation of experience, load-bearing capacity, implementation of work results, full use of technical equipment, use of advanced equipment, encouraging participation in training, and the level of special knowledge of examination theory and methodology.

Time management and work performance

Cross Ogohi Daniel, Jiya N. Santeli (Dr. Cross Ogohi Daniel, 2020) investigated the main objective of their study is to examine the effects of time management on employees' performance. The specific objectives are to determine the impact of effective time management on employee performance in Northern Nigeria Noodle Company and identify the factors that influence effective time management on employee performance in Northern Nigeria Noodle Company Ltd. Effective time management not only affects the productivity of your employees but also helps to cope with stress, conflicts and pressure more efficiently. It also helps them maintain a healthy work-life balance and keeps them motivated. The findings of the study reveal that there is a positive relationship between organizational performance and effective time management (Cross Ogohi Daniel, EFFECTIVE TIME MANAGEMENT ON EMPLOYEE PERFORMANCE , 2020). According to the literature review, we hypothesized as below:

H2. Time management positive related on work performance.

- *Impact of analysis features on workload*
- *Effect of emergency work other than main work*
- *Ability to plan work*
- *Ability to complete critical tasks on schedule*
- *Personal organization*

Attitudes and work performance

Imran Khan, and Rauqir Ahmad Ghauri (Imran Khan, 2014), studied attitude impacts on employee performance in the textile industry. Their study includes the attitude-related factors (behaviours of employees and leaders, job satisfaction, job commitment, motivation and training) to investigate their impact on employee performance. A self-administered questionnaire was used to collect the data from the textile sector of Punjab, Pakistan with a response rate of 83%. The result shows that all attitude-related factors positively affect employee performance.

Motivation and job commitment have a highly significant impact on the performance of employees. As a result, organizations should value their experienced personnel and devise effective retention policies by giving competitive salaries, experienced base pay and experienced-based promotion. That will increase the overall performance of the organization (Imran Khan, 2014). According to the literature review, we hypothesized as below:

H3. Attitude positive related on work performance.

- *Professional ethic*
- *Respect for human rights*
- *Acceptance of others*
- *Counselling and guidance cooperation*
- *Self-confidence and self-esteem*

Technique equipment and work performance

Ashima Aggarwal (Ashima Aggarwal, 2017), studied that performance appraisal system is used in organizations to measure the effectiveness and efficiency of their employees. Performance Appraisal system is needed because every employee has a different attitude to handle the work. A performance Appraisal tends to improve work performance, and communication expectations, determining employee potential and aiding employee counselling. In this paper, we present the a review of some popular performance appraisal techniques along with their pros and cons (Ashima Aggarwal, 2017). According to the literature review, we hypothesized as below:

H4. Technical equipment positive related on work performance.

- *The quality of technical equipment specifications*
- *Ability to use technical equipment*
- *Supply of hardware*
- *Availability of technical equipment*
- *Formation of technical equipment standards environment*

Experience and work performance

Micheal A McDaniel and others studied and summarized quantitative data on the relation between job experience and job performance from a total sample of 16,058 (Michael A. McDanielFrank, 1988). Work experience is occupational and industry-specific rather than firm-specific and leads to improvements in employees' job-related outcomes. They collected their study decision rules resulting in 947 samples with a total sample size of 16,058 (Nishant UppalNeharika, 2014). According to the literature review, we hypothesized as below:

H5. Experience positive related on work performance.

- *Years of work experience*
- *Experience using techniques*
- *Accumulated knowledge becomes an experience*
- *Conclusions are confirmed by experience*
- *Community learning and sharing*

We explained how professional skills, attitude, time management, technical equipment, experience related to leadership who are administrative, and executive employers work in the National Forensic Agency of Mongolia. The conceptual model of factors on managerial leadership is drawn in Figure 1.

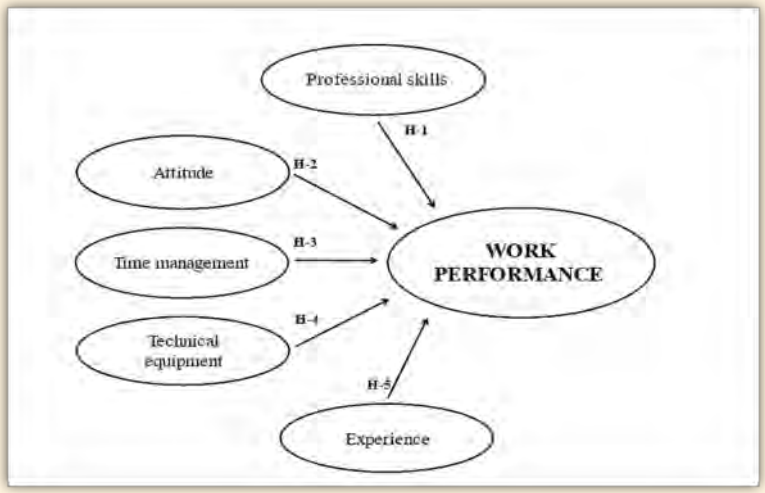


Figure 1. Source: Own diagram.

Research Methodology: Data collection and questionnaire design

We supported the previous study such as designed for using research methodology and some scholars' frameworks as below:

This study used Likert five-point scales to make it possible to discriminate opinions more finely and restrict for chosen more rather than other scales (Figure 2). Cooper (1998) described that most causal research relies on designed experimentation and simulation programs (Cooper, 1998). There are many software programs used to process data analysis. In this paper, SPSS and SmartPLS-3.0 were chosen for their simplicity and completeness (Bayasgalan TsogtsurenGankhukeg, 2022).

The Cronbach Alpha testing[1] will be used as it is the most well-accepted reliability test tool applied by social researchers. Cronbach (1946) identified that in Cronbach's Alpha reliability analysis, the closer Cronbach's Alpha to 1.0, the higher the internal consistency reliability (Bayasgalan TsogtsurenGankhukeg, 2022).

Of all the 46 respondents who fully-trained forensic signature experts were working on our research, frequency distributions were of the National Forensic Agency of Mongolia.

Processing of research results

The study included 46 experts who were or are currently conducting forensic signature examination of forensic organizations, and the results of qualitative research, factor analysis, quantitative analysis, correlation analysis, and path analysis were developed. It includes:

The results of the quantitative analysis are the crucial part of this research work, and it is part of developing, comparing, analyzing, confirming and concluding the research results.

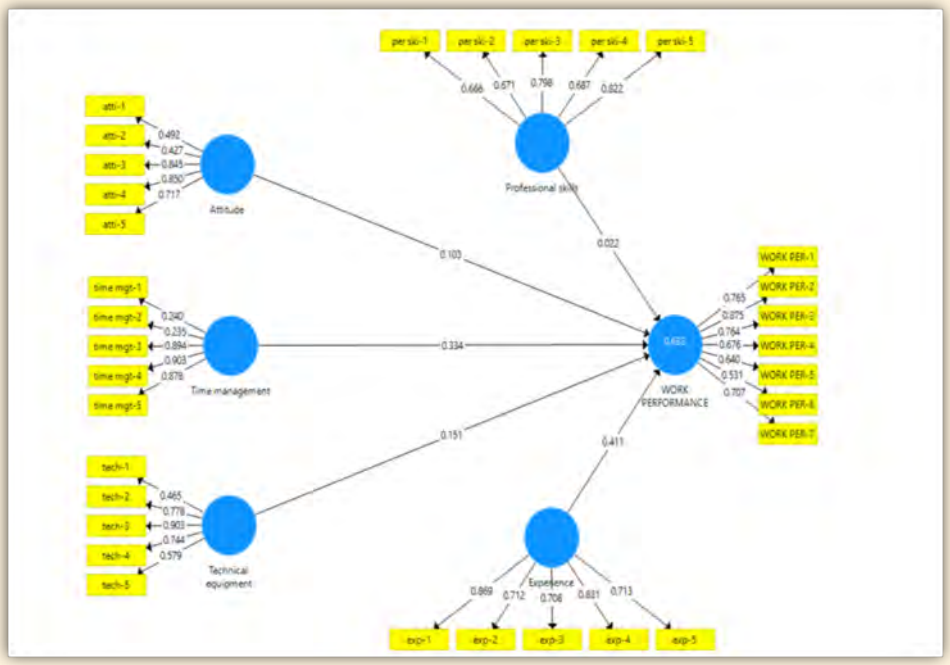
The analysis shows how each latent variable affects work performance, and the results of each factor are presented.

Formula #1. $$\alpha = \frac{N\grave{e}}{\breve{U}+(N-1)\check{c}}$$

Explanation of formula: N-number of hidden variables, $\breve{U}$ - average variance, č-average covariance

For our research work, the factor and correlation analysis of the latent variables was analyzed by trying to confirm the hypothesis by seven variables in 6 ways.

[1](*Cronbach alpha test is a measure of the reliability or internal consistency of a set of items that are supposed to measure the same concept. It is often used to assess the quality of questionnaires, surveys, or tests that have multiple items. It can also be seen as the average of all possible split-half reliabilities*)



Noted: per ski- professional skills, atti-attitude, time mgt-time management, tech-technical equipment, exp-experience, WORK Per-Work performance

Figure 2. Results of Structure Analysis of work performance (algorithm).

Factor	*item*	*Results of item*	*Cronbach's alpha*	*CR*	*AVE*
Professional skills	*Good decision-making skills*	0.666	0.784	0.792	0.536
	Use of forensic examination methods	0.671			
	Observantness and prudence	0.798			
	Thinking skills	0.687			
	Skills which write examination conclusions	0.822			

Notes: per ski- professional skills

Table 1. List of items of professional skills for each Construct of respondents

In table 1, professional skills of 5 items measuring ranged from **0.666**-**0.822**, Cronbach's Alpha of **0.784,** Composite Reliability (CR) of **0.792,** Average Variance Extracted (AVE) was **0.536.**

Factor	*item*	*Results of item*	*Cronbach's alpha*	*CR*	*AVE*
Attitude	*Professional ethic*	0.492	0.709	0.809	0.475
	Respect for human rights	0.427			
	Acceptance of others	0.845			
	Counselling and guidance cooperation	0.850			
	Self-confidence and self-esteem	0.717			

Notes: atti-attitude

Table 2. List of items of attitude for each Construct of respondents

In table 2, attitude of 5 items measuring ranged from **0.427**-**0.850**, Cronbach's Alpha of **0.709,** Composite Reliability (CR) of **0.809,** Average Variance Extracted (AVE) was **0.475.**

Factor	item	Results of item	Cronbach's alpha	CR	AVE
Time management	Impact of analysis features on workload	0.240	0.713	0.799	0.500
	Effect of emergency work other than main work	0235			
	Ability to plan work	0.894			
	Ability to complete critical tasks on schedule	0.903			
	Personal organization	0.878			

Notes: time mgt-time management

Table 3. List of items of time management for each Construct of respondents

In table 3, time management of 5 items measuring ranged from **0.235**-**0.903**, Cronbach's Alpha of **0.713,** Composite Reliability (CR) of **0.799,** Average Variance Extracted (AVE) was **0.500.**

Factor	item	Results of item	Cronbach's alpha	CR	AVE
Technical equipment	The quality of technical equipment specifications	0.465	0.832	0.829	0.505
	Ability to use technical equipment	0.778			
	Supply of hardware	0.903			
	Availability of technical equipment	0.744			
	Formation of technical equipment standards environment	0.579			

Notes: tech-technical equipment

Table 4. List of items of technical equipment for each Construct of respondents

In table 4, technical equipment of 5 items measuring ranged from **0.465**-**0.903**, Cronbach's Alpha of **0.832,** Composite Reliability (CR) of **0.829,** Average Variance Extracted (AVE) was **0.505.**

Factor	item	Results of item	Cronbach's alpha	CR	AVE
Experience	Years of work experience	0.869	0.825	0.878	0.592
	Experience using techniques	0.712			
	Accumulated knowledge becomes an experience	0.708			
	Conclusions are confirmed by experience	0.831			
	Community learning and sharing	0.713			

Notes: exp-experience

Table 5. List of items of experience for each Construct of respondents

In table 5, experience of 5 items measuring ranged from **0.708-0.869**, Cronbach's Alpha of **0.825,** Composite Reliability (CR) of **0.878,** Average Variance Extracted (AVE) was **0.592.**

Factor	item	Results of item	Cronbach's alpha	CR	AVE
WORK PERFORMANCE	Appreciation of experience	0.765	0.837	0.878	0.512
	Load-bearing capacity	0.875			
	Implementation of work results	0.764			
	Full use of technical equipment	0.676			
	Use of advanced equipment	0.640			
	Encouraging participation in training	0.531			
	The level of special knowledge of examination theory and methodology	0.707			

Notes: WORK Per-Work performance (Appreciation of experience, load bearing capacity, implementation of work result, full use of technical equipment, use of advanced equipment, encouraging participation in training, and the level of special knowledge of examination theory and methodology)

Table 6. List of items of work performance for each Construct of respondents

In table 6, technical equipment of 7 items measuring ranged from **0.531**-**0.875**, Cronbach's Alpha of **0.837,** Composite Reliability (CR) of **0.878,** Average Variance Extracted (AVE) was **0.512.**

Hypothesis	Mean	Standard deviation	T Statistic	P value	Remarks
H1. Professional skills positive related on work performance.	0.015	0.127	0.173	0.863	No supported
H2. Attitude positive related on work performance.	0.163	0.159	0.647	0.518	No supported
H3. Time management positive related on work performance.	0.266	0.190	1.754	0.078	No supported
H4. Technical equipment positive related on work performance.	0.162	0.174	0.863	0.388	No supported
H5. Experience positive related on work performance.	0.427	0.138	2.989	0.003	Supported

Notes: The result of study

Table 7. Estimated Path Coefficients of respondents on work performance.

In table 7, Hypothesis 1 such as professional skills have no related-on work performance (mean 0.015), (Standard deviation 0.127), (T statistic 0.173) and (P value 0.863). Hypothesis 2 such as attitude has no related-on work performance (mean 0.163), (Standard deviation 0.159), (T statistic 0.647) and (P value 0.518). Hypothesis 3 such as time management has no related-on work performance (mean 0.266), (Standard deviation 0.190), (T statistic 1.754) and (P value 0.078). Hypothesis 4 such as technical equipment has no related-on work performance (mean 0.162), (Standard deviation 0.174), (T statistic 0.863) and (P value 0.388). Hypothesis 5 such as experience has no related-on work performance (mean 0.427), (Standard deviation 0.138), (T statistic 2.989) and (P value 0.003).

Conclusion

We studied in the fiscal year of 2022 our paper collected and delivered an online form- questionnaire with an official inquiry that requested quantitative and qualitative surveys in our study. There are participated 18 experts who work in the National Forensic Agency of Mongolia, 13 experts who work Forensic department of the capital city, and 15 experts in who Rural Forensic department in our study. We were hypotheses five hypotheses. One of them is supported and four of them are not supported in path analysis.

We are recommending our study as below:

a. To study more hypotheses, and result in the future.
b. To study and compare factors on work performance with another special agency.
c. To study and compare the factors with foreign scholars' studies in the future.

Finally, we will study our next research paper, which needs to correlate skills, leadership, job satisfaction, engagement, behaviour with performance management and etc.

References

[1] *Ashima Aggarwal, G. T. (2017). Techniques of Performance Appraisal. Semantic Scholar, 254-268.*

[2] *Bayasgalan Tsogtsuren, G. G. (2022). THE ANALYSIS IMPACTS OF EMPLOYEE'S LEADERSHIP AT NATIONAL FORENSIV SCIENCE INSTITUE OF MONGOLIA. International Journal of Innovation Scientific Research and Review, Vol. 04, Issue, 02, pp.2344-2349.*

[3] *Claire Johnson, P. S. (2006). Leitch Review of skilss. Final report, Prosperity for all in the global economy-world class skills. The Stationery Office 1-153.*

[4] *Cooper, H. (1998). Synthesizing research: A guide for literature 3rd ed. Oaks: CA: Sage Publications, Thousand.*

[5] *Cross Ogohi Daniel, S. J. (2020). EFFECTIVE TIME MANAGEMENT ON EMPLOYEE PERFORMANCE . INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT, 72-84.*

[6] *Cross Ogohi Daniel, S. J. (2020). EFFECTIVE TIME MANAGEMENT ON EMPLOYEE PERFORMANCE OF NORTHERN NIGERIA NOODLE COMPANY LTD. INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT .*

[7] *Dr. Cross Ogohi Daniel, D. J. (2020). EFFECTIVE TIME MANAGEMENT ON EMPLOYEE PERFORMANCE OF NORTHERN NIGERIA NOODLE COMPANY LTD . INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT, p.72-82.*

[8] *Grugulis, I. a. (2011). Skill and Performance. British: British Journal of Industrial Relations, Vol. 49, Issue 3, pp. 515-536, 2011.*

[9] *Imran Khan, H. D. (2014). Impact of Attitude on Employees Performance: A Study of Textile Industry in Punjab, Pakistan. World Applied Sciences Journal, 191-199.*

[10] *Michael A. McDaniel, F. L. (1988). Job Experience Correlates of Job Performance. Journal of Applied Psychology, Vol. 73, No. 2,327-330.*

[11] *Nishant Uppal, N. V. (2014). Prior Related Work Experience and Job Performance: Role of personality. International J Journal of Selection and Assessment, 39-44.*

[12] *Rhoades, P. C. (1983). Role Performance and Person Perception: Toward an Interactions Approach. Wiley, Vol. 6, No. 2 (Fall 1983), pp. 207-227.*

[13] *Stephen Machin, S. M. (2001). Basic Skills, Soft Skills and Labour Market Outcomes: Secondary Analysis of the National Child Development Study. 1-45.*

[14] *Stoyanova, I. G. (2011). Skill and Performancebjir_779 515..536. British Journal of Industrial Relations, doi: 10.1111/j. 1467-8543.2010.00779. 2011 0007–1080 pp. 515–536.*

[15] *Imran, A. (2019). Personality traits, individual innovativeness and satisfaction with life. Journal of Innovation and Knowledge , 38-46.*

[16] *Janie, B. B. (2006). Ethics in Organizations and Leadership. Jones and Bartlett .*

[17] *Klingborg, D. J. (2014). What Is Leadership? Leadership and Professional Development, 280-285.*

[18] *Mas-Machuca, M. (2014). The Role of Leadership: The Challenge of Knowledge Management and Learning in Knowledge-Intensive he Role of Leadership: The Challenge of Knowledge. International Journal of Educational Leadership and Management, 97-116.*

[19] *Mihelič, K. K. (2010). Ethical leadership. International Journal of Management and Information Systems, 31-44.*

[20] *Sharma, R. T. (n.d.). Managerial Skills for Managers in the 21st Century.*

[21] *Singh, S. K. (2008). Role of leadership in knowledge management: A study . Journal of Knowledge management , 3-15.*

[22] *Smuthy, P. (2012). The Relationship between Managerial Skills and Managerial Effectiveness in a Managerial Simulation Game . Etrategia Organizaion Czech Science Foundation, 11-21.*

[23] *Stogdill, R. (1948). Personal factors associated with leadership: A survey of the literature. Psychol, 64.*

[24] *WK Hoy, C. M. (1987). Educational Administration:Theory, Research, and Practice 3rd ed. New York: Random House*

Analysis of Fonts in Questioned Documents

Ms Wan Rahimah Wan Ahmad, Ms Siti Nur Musliha Mohamad Nor, Ms Nurul Atiqah Mohd Noh,*
Mr Muhammad Rafiuddin Jailani,
Forensic Science Analysis Centre, Department of Chemistry, Malaysia
**Email : wanr@kimia.gov.my*

Abstract

Font forgery is a type of fraud that occurs when a computer, rather than a typewriter, is used to store or prepare a document. According to Malaysian law, "anyone who falsifies a document for the purpose of cheating has committed an offence under Section 468 of the Penal Code (Act 574), which can result in up to seven years in prison and a fine if convicted." In Malaysia, there have been several cases involving font analysis such as compliance of the Act in Malaysia "Direct Sales and Anti-PYRAMID SCHEME ACT 1993 (ACT 500) AND REGULATIONS, determining whether the font, size, spacing on the disputed document is the same as the specimen sent by one of Enforcement Agency in Malaysia and Determination of font type printing on disputed documents. This write-up suggests a few factors that should be considered when analyzing a document's typeface since they could indicate a new kind of fraud to a document examiner.

Introduction

What is a font?

A font is a set of printable or displayable typography or text characters in a specific shape that are available in printed or digital form. A typeface is the abstract design of one or more fonts, as a family, regardless of format.

Typography is the ability to combine typeface, font, and spacing to create designs for products such as websites, brochures, books, and computer graphics. The ability to create an effective and aesthetically acceptable design depends entirely on the graphic designer or typographer.

In contrast to the graphic designer, the Forensic Document Examiner (FDE) is one of the forensic science disciplines where expert examiners assess the disputed documents in the legal system with the aim of methodically evaluating attributes and characteristics of a document's conciseness to reveal how it was prepared or how it may have been modified.

One of the FDE's areas of expertise in the field of forensic document examination is comparative typography analysis, which is used to verify the legitimacy of a document by comparing it to both the original and a disputed document with appropriate document specimens for comparison. When the font used in the document predates the design of the typeface by a font designer, this is an example of forgery in a forensic document and is called an anachronism [1]. Hence, the expertise of both typeface designers and document examiners working together can create evidence of whether a document is genuine or not.

Relationship between Font, Font Family, and Typeface.

Typeface is the shape and general style of letters, for example, Times New Roman, Arial, and Calibri. Times new Roman, Arial and Calibri are the names of typefaces with different shapes, whereas 20 pt is the size unit for the font referred to as point in units. A font family is a group of multiple related styles of a typeface, such as Arial Black, Arial Bold, and Arial Round Mt Bold.

Background

Categories of fonts

Although there are other categories, three common categories of fonts are: serif, sans serif (without serifs), and script. The term "serif" refers to the stroke or decorative line or taper added to the beginning or end of a letter's stem, while sans serif (without serif) is a typeface style that uses straightforward and uncluttered lines. Script font is a writing style that employs connecting strokes to achieve the desired appearance of elegance and grandeur. This style of typeface is widely used for invitation cards, announcements, and attractive initials.

The Anatomy of Types

The visual components that make up a typeface's letterforms are referred to as its anatomy. Each letterform is composed of various parts of the font terminology, such as the spine, stem, and stroke. When type designers build typefaces, components are essential elements that influence a typeface's overall appearance and readability. Further illustrations related to font terminology can be found in the book by Stephen Coles, "The Anatomy of Type".[2]

It is recommended that forensic document examiners understand some fundamental elements of font parameters, such as kerning, leading, and tracking. Kerning is an adjustment to the space between two individual letters, usually based on data about that combination that is built into the font; tracking is using a single global adjustment to the space between all the letters in a body of text. Leading is the vertical distance between the baselines of consecutive lines of text, or the amount of adjustment to that distance. Books written or published by

professionals in the field of typography can be used as a reference for other important basic terms for fundamental typography matters, such as glyph, x-height, and cap height.

Materials and methods

Typography E Ruler

A typography *E* ruler is the tool used to measure a close approximation of the font size on a printed page document, as suggested in the reference book "Scientific Examination of Questioned Documents", second edition, edited by J.S. Kelly and Brian S. Lindblom [3].

For the purpose of this exercise, a typography E ruler was purchased online from Galaxy Gauge, Colorado, USA. It has the letters E and x printed on it (with two types of fonts: serif and sans serif) and unit sizes in millimeters, centimeters, inches, and picas, which are sequenced from 6 point to 72 point of size. It was historically used in the graphic arts to check the dimensions of typographic materials. A unit can be converted to a more precise unit by using a conversion factor, such as 72 pts equal to 1 inch, 12 pts equal to 1 pica, and 6 picas equal to 1 inch.

This tool is for getting measurement of font leading but it should not be used for font size if a high level of accuracy is required. These two measurements are always expressed on the vertical axis and expressed in terms of points. This ruler by Galaxy gauge is made of a low thermal expansion substrate and is traceable to the National Institute of Standards and Technology.

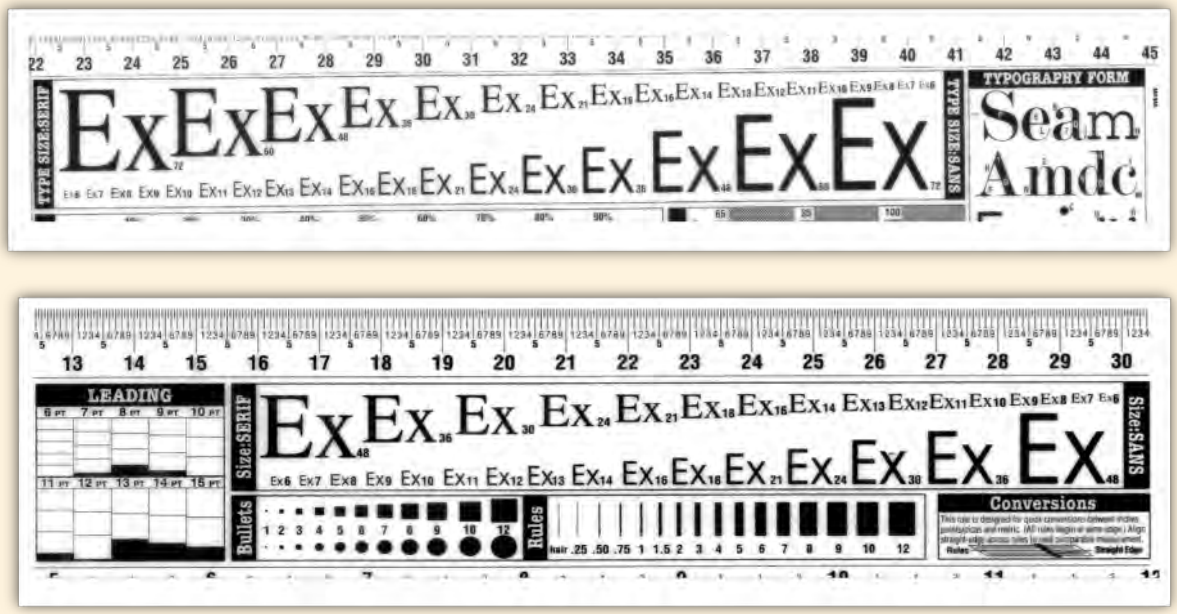


Figure 1. A picture of a typography E-ruler purchased from Galaxy Gauge, Scientific Illustration Service, Colorado, USA.

Measurement using an E-ruler

Determination of font size

Determine the class characteristic, either sans-serif or serif, for the printed document. The printed section "Ex" on the e-ruler was overlaid on the printed document correspondingly (serif or sans serif). The font size printed on the e-ruler was read directly by referring to the vertical height of the "E" on the e-ruler, which matched the font size of the capital letter on the printed document.

For the document examiner, this measurement is an approximate measurement of the font size to render an opinion.

Figure 2a

Figure 2b

Figure 2a. The "Ex" on the E-ruler was placed next to the capital letter of "RM" to get the closest font size (point).

Figure 2b. The "Ex" on the e-ruler was overlaid above the capital letter of "RM" to get an approximate font size (point).

Determination of line spacing (leading)

The E-ruler was laid over the printed documents; the wording for each line should be fitted and correspond to the provided space on the E-ruler.

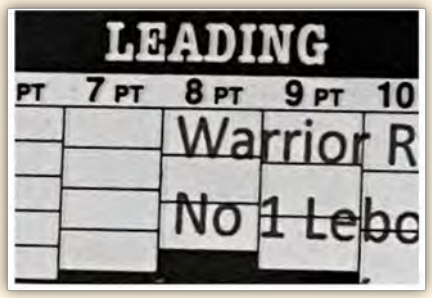


Figure 3a

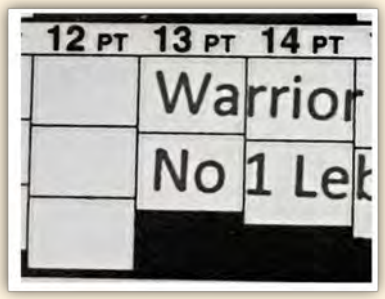


Figure 3b

Figure 3a. The word "Wa" is in the space, however the word "No" is not.

Figure 3b. The capital letter "Wa"(first line) and "No"(second line) in a space corresponding to point size 13. Hence, the spacing (leading) for the sentence was approximately 13 points. (All lines of text must be separated by spaces).

Examination using Video Spectral Comparator (VSC)

From the point of view of a document examiner, most questioned documents are examined using VSC equipment. The main function of VSC is to use magnification and lighting techniques. For cases involving font determination, comparison specimens need to be provided together for comparison purposes. VSC is used to see these design characteristics of the font. Similarities and differences in font design characteristics are observed and evaluated.

Figure 4a

Figure 4b

Figure 4a. A superimposed comparison between the questioned document (red) and the specimen document (green) using VSC.

Figure 4b. The similarity of design characteristics between the questioned font "Q1-A" and the specimen font "S". The vertical of the small letter "l" is higher than the capital letter "F."

Results and Discussions

As a Forensic Document Examiner, the measurement by typography E ruler is an aid tool to measure only a very approximate size of font. Therefore, the measurement results obtained cannot be used when an actual font size needs to be determined. Other points to be considered when measuring the font size of printed documents are printing output and specimen's media. It may differ slightly depending on the type of printer and printing method used and should be performed on printed documents. The printed document submitted will be considered an original document. Physically measuring type size on screen is ineffective for determining any inherent point size in a digital document because it varies depending on screen size and resolution without changing the document. As a result, if the disputed document is in digital form, it will be referred to the relevant agency.

Conclusions

For forensic document examiners, it is recommended that they learn the basics of typography by attending training provided by typography experts. For forensic document examiners, it is recommended that they learn the basics of typography by attending training, workshops, and other related courses. Training on typography E-ruler should be focused on the fundamentals of typography measurement techniques, whereas E-ruler is one of the aid tools suggested for the measurement of font produced by printing documents. This is because it differs from the font size reading from computer word processing software, and to render an opinion, the font can be approximately measured using an e-ruler.

Basic document examination tools such as microscopes and high-end instruments such as Video Spectral Comparator (VSC) can be applied for the examination of fonts with the use of an integrated combined system made up of cameras and flood light sources, assisting in the accurate comparison of questioned documents.

The E-ruler mentioned in this article is a tool that a document examiner may use to obtain faster and more accurate results from examinations for new cases, such as fonts. This article provides guidelines for the document examiner to get approximate measurements of the font by using the E ruler to render an opinion; however, if the precise font size needs to be determined, it should be obtained from a typography expert.

Acknowledgment

This article has been reviewed by two key individuals throughout our font training. We would like to express our gratitude to Mr. Thomas Phinney, a Typography Specialist, and Mr. Michael Wofsey, Principal Researcher at Galaxy Gauge, USA.

References

[1]. Loganathan Lingan, Anachronism in Fonts and Relative Dating of Computer, Arab Journal of Forensic Sciences & Forensic Medicine Printed Documents: A Case Report, 17 June 2020, ASFSFM 2020; Volume 2 Issue (2) Received 23 Feb. 2020;Accepted 07 June. 2020; Available online 07 Sep. 2020, 6 pages.*

{2]. Stephen Coles, "The Anatomy of Types. A graphic Guide to 100 typeface", Third Printing 2021, 195 Broadway, New York, NY 10007, 2021.

[3]. J.S Kelly, Brian S.Lindblom "Scientific Examination of Questioned Documents", Second Edition, Broken Sound Parkway,CRC Press 2006

[4]. John D. Berry, John Hudson, "Now Read This, The Microsoft Cleartype Font Collection" , Microsoft Corporation, 2004

[5] Formal Script-detailed Information on the Font: Category free, TTF, Microsoft Word, Photoshop, Corel Draw, adobe illustrator, autocad, Sony reader, adobe Sony reader

The Absorption Kinetic of Black Hair Contaminated by Benzodiazepines in Exogenous Blood/Urine

Bo Zou[1], Jing Chang[1], Aihua Wang[1], Yunfeng Zhang[1], Jifeng Wu[2]*
1. Institute of Forensic Science, Ministry of Public Security, China.
2. Institute of Criminal Sciences and Technology, Ji'nan Bureau of Public Security, China.
The People's Republic of China
** Email: zoubo1141@126.com*

Abstract

In the case of direct contact, benzodiazepines in blood and urine showed spontaneous enrichments into black hair, causing irremovable contaminations. This permeation started almost immediately, and took 4–5 days to reach adsorption equilibrium. The equilibrium adsorption concentrations of 8 benzodiazepines into hair were 2.4–4.9ng/mg from the blood containing 1.0μg/mL benzodiazepines at room temperature. The adsorption capacity of benzodiazepines in hair was proven to be concentration dependent. As individual variability, gender and cosmetic treatment both affect the drug adsorption characteristics. This study was to deepen the understanding of hair contaminated by drug containing body fluids, and provide reference for the assessment of drug exposure (from body and environment) through hair samples.

Introduction

Hair has proven to be an effective biological specimen for assessing drug and toxicant exposure. In the cases of anesthesia sexual assaults with delayed reporting[1], sampling tests of drug abuse and decomposed body detections[2], hair toxicology analysis often provided valuable court evidence. Hair cleaning procedure with solvent and surface active agent is usually necessary. However, it is still difficult to identify and eliminate the contaminations of hair, such as endogenous drug from sweat or urine, and exogenous drug smoke and powder from the environment. In a simulated contamination study, direct contact of hair with external cocaine could made subjects indistinguishable from actual users[3]. Besides, soakage of blood at the crime scene and impregnation of decayed bodies may also negatively impact the hair drug analysis.

Benzodiazepines were frequently used in clinical treatments and drug-facilitated crimes (DFC). Hair analysis have been used to confirm the benzodiazepine exposure[4], including intake time extrapolation[1]. In the in-vitro model of Claffey[5], a moderately irreversible interaction was proven between ^{3}H-flunitrazepam and hair melanin. In our research, blood and urine containing benzodiazepines were used as exogenous contaminants to observe the binding of drugs against black hair matrix. We investigated the kinetic characteristics of benzodiazepines permeation into hair, and discussed the influencing factors of adsorption capacity. We hope to deepen the understanding on the mechanism of drugs binding into hair, and provide reference for the interpretation of hair toxicant detection.

Materials and methods

Biological Samples and chemicals

Black hair samples, blood and urine were collected from 8 healthy volunteers (no exposure to benzodiazepine within 6 months). Triazolam, diazepam, clonazepam, 7-aminoclonazepam, estazolam, midazolam, alprazolam and α-hydroxyalprazolam in 1.0 mg/mL standard solutions were purchased from First Standard® (Tianjin, China). Ultrapure water and chromatographic methanol (LiChrosolv®, Germany) were used. Instruments used in this study were: ball mill (Wondfo®, Beijing, China), electronic balance (Mettler Toledo®, Switzerland) and HPLC-MS/MS (AB Sciex 4000 Qtrap®, USA).

Hair contamination

About 20 mg hair was washed with water and methanol, and soaked in 1.0 mL blood/urine containing increasing concentrations (0.01–10 μg/mL) of benzodiazepines respectively. The hair was taken out regularly (within 0–5 days) and washed repeatedly with water and methanol, until the benzodiazepine in the last washing solution was below 0.1% of the soaking solution (detected on HPLC-MS/MS). The hair was then dried in the air, accurately weighed, ground into powder and extracted with 1.0mL methanol for HPLC-MS/MS analysis. Hair without contamination was added with benzodiazepine standards (0.001–1μg), ground into powder and extracted with 1.0mL methanol, as quantitative external standards for HPLC-MS/MS.

Instrumental conditions

Chromatographic separations were performed on Agilent Zorbax Eclipse Plus C18 column (2.1×50 mm, 1.8 μm in particle size), with a total flow rate of $0.8 \cdot mL/min^{-1}$ at 30°C. 0.1% formic acid aqueous solution (A) and methanol (B) were used as mobile phase.

The ion source of MS/MS: ESI^{+}; Acquisition mode: multiple reactions monitoring (MRM). Ion source temperature:500°C; Curtain gas: 20 psi; Ion spray voltage:4.5 kV.

Results and discussions

A regular monitor was carried for the continuous penetration of 1.0 μg/mL diazepam from blood to hair (Figure 1). An irreversible binding of diazepam with hair matrix was observed only after 10 minutes, and it

took about 4~5 days to reach the adsorption equilibrium, with 4.8ng/mg diazepam in the hair. In terms of mass concentration, the spontaneous enrichment of diazepam to hair is quite obvious. A contrast experiment showed more diazepam accumulated into hair from urine than from blood (Figure 2), which was also confirmed with estazolam and clonazepam. This may be attributed to the lesser protein and pigment in urine, which were considered to provide binding sites for drugs. We believe it is of a great risk to speculate benzodiazepine intake time through segmented detection of pubic and underarm hair.

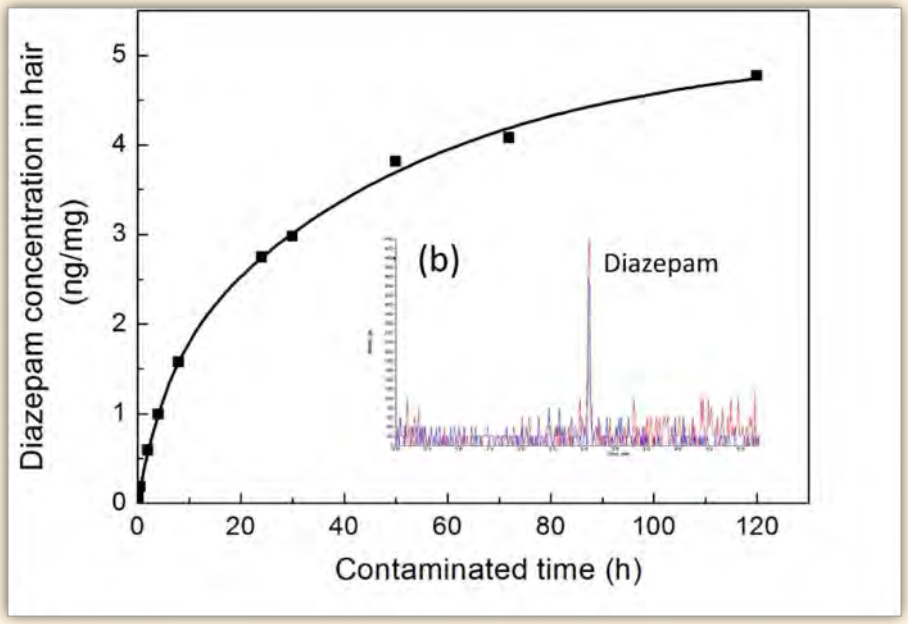


Figure 1 (a). The curve of diazepam adsorption into hair. (b) The XIC of hair contaminated for 10 min.

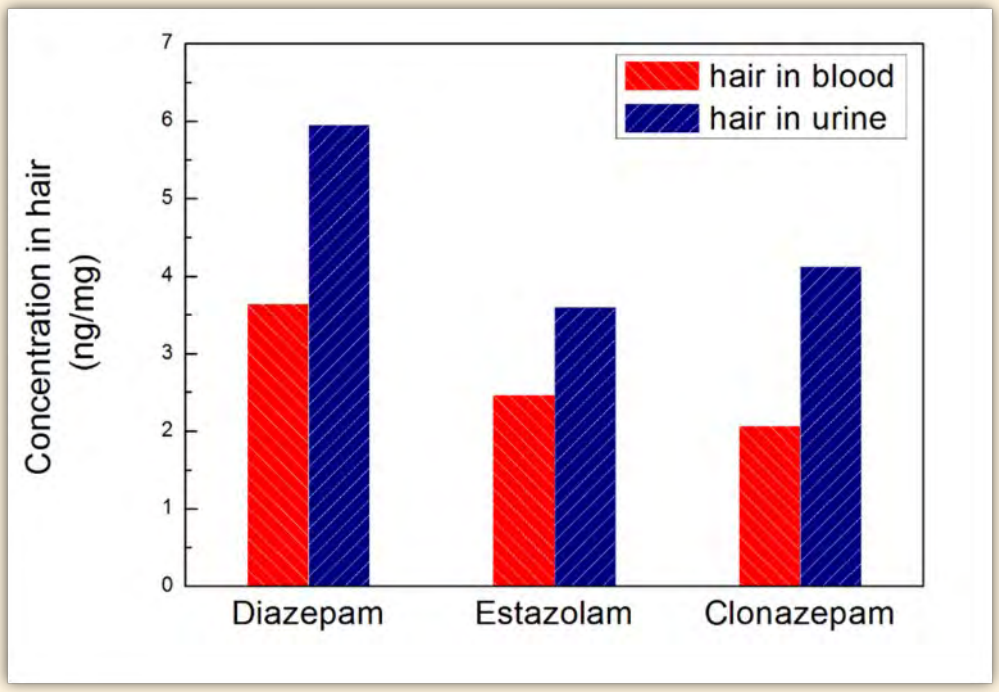


Figure 2. Comparison of hair contaminations by blood and urine containing 2.0μg/mL benzodiazepine for 16 h in room temperature.

Afterwards, we investigated the concentration dependence of benzodiazepine adsorption. Blood samples containing 0.01–10.0μg/mL benzodiazepine were used to soak the hair in parallel. For the same drug，the hair-blood concentration ratios were found to be very close (Table 1), which conformed to the law of passive diffusion between two phases. Therefore, the contaminated hair samples from bloody cases or decomposed bodies may be valuable to estimate the content level of benzodiazepines in external contamination source. We carried out a comparative study on 8 benzodiazepines and listed the equilibrium adsorption capacities in 1.0μg/mL blood (Figure 3). The spontaneous enrichment to hair was rather common for benzodiazepines, in which diazepam and midazolam showed the highest contents, being 4.6 ng/mg and 4.9ng/mg respectively. Relative to clonazepam and alprazolam, their primary metabolites, 7-aminoclonazepam and α-hydroxyalprazolam, were 10-20% lower in adsorbed concentrations. The possible cause is the stronger polarity of metabolites influences the binding affinity against hair matrix. In 2003, Scott[6] reported that the incorporation rates (ICRs) of benzodiazepines into rat hair were significantly different after intraperitoneal injection. In our research, no such significant difference was observed on equilibrium adsorption capacities in external contact for 8 benzodiazepines including the ones with diazole and triazole. It may be implied that the selective adsorption of basic and liposoluble drugs into hair mainly occurs inside of the hair follicle, affected by the pH value of hair matrix cells.

	benzodiazepine	Soaking time (h)	Benzodiazepine concentration in blood (μg/mL)			
			0.01	0.1	1.0	10
Concentration in hair (ng/mg)	Clonazepam	18	<LOQ	0.110	1.20	10.1
	Triazolam	18	0.024	0.116	1.33	10.6
	Estazolam	12	<LOQ	0.090	0.78	8.13
	Alprazolam	12	<LOQ	0.056	0.50	5.16

Table 1. Effect of external drug concentration on hair adsorption.

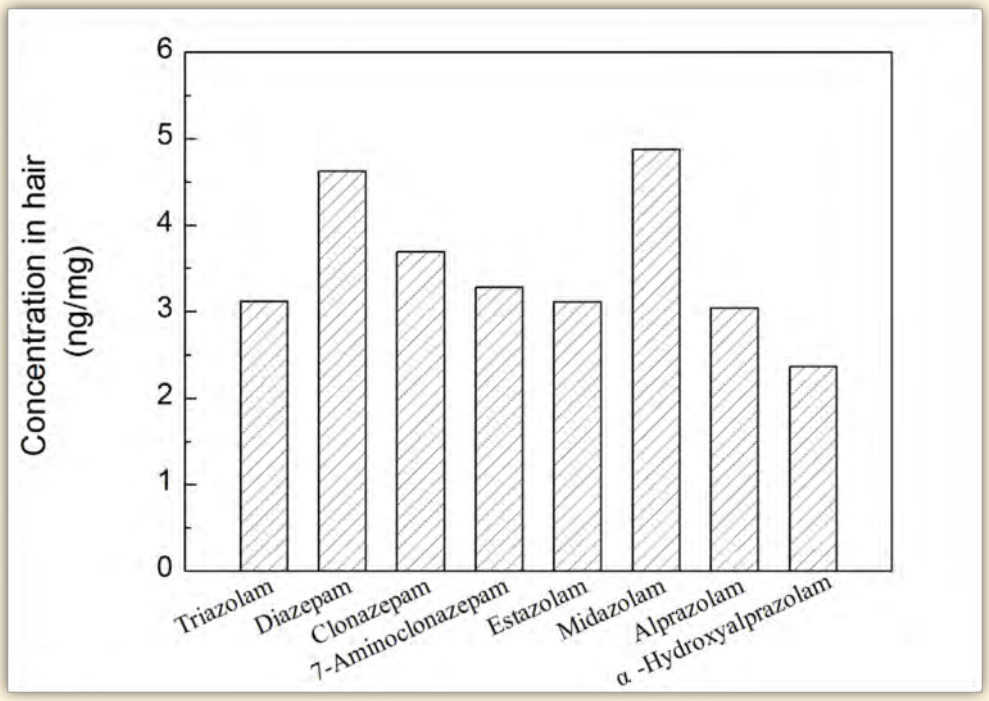


Figure 3. Concentrations of 8 benzodiazepines in the hair soaked in blood samples containing 1.0μg/mL of the benzodiazepines separately (after 4 days at room temperature).

Hair samples from 8 volunteers (V1~V8 in Table 2) were compared on triazolam adsorption. The adsorbed concentration in female hair (V6~V7) samples were observed to be slightly higher than male (V1~V5), probably due to the smaller diameter of female hair, which means larger contact surface of hair with urine or blood containing benzodiazepines and faster drug adsorption. The dyed brown hair (V8) showed almost double absorption capacity in the same period of time, indicating a significantly increase on hair surface penetrability. Considering drug penetration is a two-way process, the dyed hair of benzodiazepine users may be more likely to lose the drugs in daily lives.

Sample	Gender	Color	Triazolam concentration in the hair* (ng/mg)	
			Soaked in blood#	Soaked in urine#
V1	male	black	0.24	0.23
V2		black	0.22	0.23
V3		black	0.19	0.24
V4		black	0.24	0.30
V5		black	0.20	0.26
V6	female	black	0.26	0.29
V7		black	0.29	0.42
V8		dyed brown	0.57	0.65

* *Soaked for 10 h at room temperature.*
\# *Containing 0.2 μg/mL triazolam.*

Table 2. Individual difference of black hair on triazolam adsorption.

Conclusions

We investigated the kinetic characteristics of 8 benzodiazepines adsorption into black hair from external blood/urine in this work. A spontaneous enrichment turned out common for benzodiazepines and the adsorption capacity was time and concentration dependent. Dyed hair seems to be more permeable to benzodiazepine molecules. In summary, we suggest caution in estimating the intake time of benzodiazepines through hair segmental analysis. On the other hand, contaminated hair from bloody cases and putrefied body may be useful to estimate the external drug concentration.

Acknowledgement

This work was supported by the basic scientific research project (2020JB010) of Institute of Forensic Science, Ministry of Public Security, China.

References

[1] *Koren G, Bellaish E, Maman K. Hair analysis for drug-facilitated crime: the critical role of hair growth rate[J]. J Forensic Sci, 2019, 54(5):1574–1575.*

[2] *Kuwayama K, Nariai M, Miyaguchi H, et al. Estimation of day of death using micro-segmental hair analysis based on drug use history: a case of lidocaine use as a marker[J]. Int J Legal Med, 2019(133):117–122.*

[3] *Paulsen R B, Wilkins D G, Slawson M H, et al. Effect of four laboratory decontamination procedures on the quantitative determination of cocaine and metabolites in hair by HPLC-MS[J]. J Anal Toxicol, 2001(25):490–496.*

[4] *Vogliardi S, Favretto D, Tucci M, et al. Simultaneous LC-HRMS determination of 28 benzodiazepines and metabolites in hair. Anal Bioanal Chem, 2001(400):51–67.*

[5] *Claffey D J, Stout P R, Ruth J A. 3H-Nicotine, 3H-Flunitrazepam, and 3H-Cocaine incorporation into melanin: a model for the examination of drug-melanin interactions[J]. J Anal Toxicol, 2001(7):607–611.*

[6] *Karen S S, Yuji N. A study into the rate of incorporation of eight benzodiazepines into rat hair[J]. Forensic Sci Int, 2003 (133):47–56.*

The Detection of Methcathinone in Urine Samples after Consumption of Ephedrine or Pseudoephedrine

Ms Goh Mei Ling Evelyn, Ms Moy Hooi Yan and Dr Lui Chi Pang*
Drug Abuse Testing unit, Analytical Toxicology Laboratory, Health Sciences Authority, Singapore
**Email: evelyn_goh@hsa.gov.sg*

Abstract

In the course of our routine casework, low levels of methcathinone, a controlled drug under our local legislation, were detected in urine samples (ranged from 1.9 to 238 ng/mL) containing high concentrations of ephedrine or pseudoephedrine (ranged from 10 to 136 µg/mL). The latter two are common ingredients that can be found in cold and flu medications. This study investigates the postulations whether methcathinone could be derived from the sample preparation procedures for amphetamines analyses or from the metabolism of ephedrine or pseudoephedrine in the human body. The results show that water and urine samples fortified with ephedrine and pseudoephedrine exhibited trace amounts of methcathinone (0.23 to 3.51 ng/mL), indicating methcathinone could be an artefact resulting from the sample preparation process. The presence of methcathinone in urine samples from routine forensic casework containing pseudoephedrine or ephedrine suggests the possibility that methcathinone could also be a result from the metabolism of pseudoephedrine or ephedrine in the body. A mechanism which generates a geminal diol as an intermediate, catalyzed by dopamine-b-hydroxylase, is postulated for the conversion of pseudoephedrine or ephedrine to methcathinone. In this study, we have demonstrated that the detection of low levels of methcathinone in urine samples is not necessarily a result of the consumption of illicit methcathinone.

Introduction

Ephedrine and pseudoephedrine are common medicines to relieve blocked nose and cough for the treatment of common cold, flu and allergies. In the routine analysis of urine samples for drugs of abuse in our laboratory, low amounts of methcathinone, ranged from 1.9 to 238 ng/mL, were detected in urine samples containing high concentrations of ephedrine or pseudoephedrine. It was suspected that the presence of methcathinone at low concentrations in urine could either arise from the sample preparation procedures or from the metabolism of ephedrine or pseudoephedrine in the human body. The aim of this study is to reveal the pathway that leads to the presence of methcathinone in the urine samples.

Material and Methods

Sample extraction study

Ephedrine (EPH) and pseudoephedrine (PEPH) certified reference materials at 1 mg/ml were purchased from Cerilliant (Round Rock, TX, USA). Stock standard solutions at 100 µg/mL were prepared in LC-MS grade methanol from the reference materials and stored at –20 °C until use. Individual neat standards of ephedrine and pseudoephedrine prepared at 10 µg/mL in methanol, and dissolved Decondine® tablet (containing triprolidine HCl 2.5 mg and pseudoephedrine HCl 60 mg) prepared at 10 µg/mL and 100 µg/mL of pseudoephedrine in methanol were analyzed as controls. Spiked samples containing ephedrine, pseudoephedrine and dissolved Decondine® tablet in water and drug-free urine at the same concentrations were also extracted and analysed.

Briefly, each urine sample (0.5 mL) was hydrolyzed using b-glucuronidase enzyme (Haliotis Rufescens from Finden Kura Biotec (Parcelacion Neumann, Puerto Varass, Chile) in ammonium acetate buffer (pH 5). The mixture was incubated in a water bath for 1 hour at 60 °C. After cooling to room temperature, the hydrolysed urine sample was adjusted to pH 11-12 using ammonia solution prior to supported-liquid extraction (Isolute® SLE+ cartridge, 1 mL). Elution was carried out twice each with 2 mL isopropanol-ethyl acetate (5:95 v/v). The eluate was acidified with 1% HCl in methanol (v/v) before evaporating to dryness and reconstituted in 500 µL water-acetonitrile (80:20 v/v) for instrumental analysis. The concentrations of methcathinone and pseudoephedrine were analysed using a liquid chromatograph tandem mass spectrometer (LC-MS/MS) with ESI positive mode on a Kinetex® XB-C18 column (2.6 µm, 2.1 x 150 mm). Gradient elution was performed using mobile phases 10 mM ammonium formate with 0.1% formic acid (A) and acetonitrile (B).

Controlled administration study

Urine samples were procured from three healthy volunteers (no flu medications were taken by the volunteers at least one week before the study was conducted) at 0, 1, 3, 5 and 8 hours after the oral administration of one Decondine® tablet. One of the volunteers took a second oral dose of one Decondine® tablet 12 hours after the first dose, and the urine samples were collected again at 12, 13, 15, 17 and 20 hours. The volunteers' urine samples were screened using Kinetic Interactions of Microparticles in Solution (KIMS) immunoassay for amphetamines on a Cobas c501 analyzer (Roche Diagnostics). Subsequently, the urine samples were extracted and analysed using the LC-MS/MS method described above.

Routine urine sample study

Thirty-seven (37) authentic urine samples encountered from routine forensic casework and containing varying amounts of ephedrine or pseudoephedrine were also extracted and analyzed for methcathinone.

Results and Discussion

Sample extraction study

Analysis of spiked water and urine samples (A, B and C) containing 10 µg/mL and 100 µg/mL of pseudoephedrine prepared using Decondine® tablet, shows the presence of 0.23 to 1.48 ng/mL of methcathinone in spiked water (Figure 1, indicated by light blue bars), and 0.43 to 2.23 ng/mL of methcathinone in spiked urine samples (Figure 1, indicated by green bars). Higher amounts of methcathinone ranged from 1.28 to 3.51 ng/mL (Figure 1, indicated by orange bars) were detected in water and urine samples spiked with 10 µg/mL of ephedrine. Separate analysis using the same LC-MS/MS method on individual neat standards of ephedrine, pseudoephedrine and the Decondine® tablet in methanol shows the absence of methcathinone *(data not shown)*. Negative water and urine control samples (without ephedrine or pseudoephedrine) were included in the spiked sample experiments, and no methcathinone was detected. Based on these results, it was suspected that methcathinone could be an artefact resulted from the sample preparation process. Furthermore, the chirality of the compound could have affected the conversion to methcathinone as indicated by the significantly higher amount of methcathinone in water or urine sample fortified with ephedrine as compared to pseudoephedrine, as shown in Figure 1.

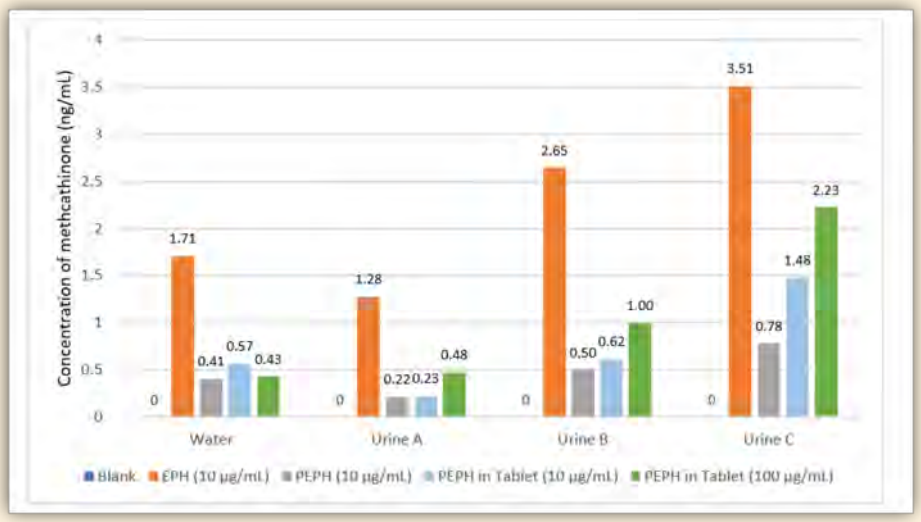


Figure 1. Concentrations of methcathinone (average of duplicates) detected in spiked samples in water and urine matrices, extracted using SLE sample preparation.

Controlled administration study

For the screening test, Table 1 shows the results of the KIMS amphetamines assay of the volunteers' urine samples. All samples, except for the urine from Volunteer 3 at 1-hour post-administration, were screened negative for amphetamines (below laboratory screening cut-off at 500 ng/mL). Based on the manufacturer's information, cross reactivity of pseudoephedrine for the KIMS amphetamines assay is 0.44%.

Time after the first dose (hour)	Volunteer 1	Volunteer 2	Volunteer 3
0	132	118	337
1	130	78	955
3	461	123	138
5	484	99	187
8	45	81	221
12	150	-	-
13	185	-	-
15	229	-	-
17	379	-	-
20	468	-	-

Table 1. Results from KIMS Amphetamines assay for volunteers' urine samples (cut-off 500 ng/mL).

In the analysis using LC-MS/MS, the results show the presence of pseudoephedrine and methcathinone in the volunteers' urine samples at various time points (Figure 2). It was observed that generally, the detection of methcathinone increased with an increase in amount of pseudoephedrine in the urine samples. After a single dose of Decondine® tablet, methcathinone could be detected up to 3.97 ng/mL (Volunteer 3, 3 hours after consumption) and pseudoephedrine up to 77.3 µg/mL (Volunteer 1, 8 hours after consumption) in the urine samples. Following a second oral dose taken by Volunteer 1, pseudoephedrine could be detected up to 85.3 µg/mL in the urine sample (3 hours after second dose). However, despite the double dosing regime, only up to 2.06 ng/mL of methcathinone (8 hours after second dose) was detected in Volunteer 1's urine sample during the study.

Figure 2. Concentrations of pseudoephedrine (line) and methcathinone (bar) detected in volunteers' urine samples after consumption of Decondine® tablet.

Routine urine sample study

Similar trend of increased detection of methcathinone with the higher amounts of pseudoephedrine or ephedrine in the 37 routine urine samples were generally observed (Figure 3).

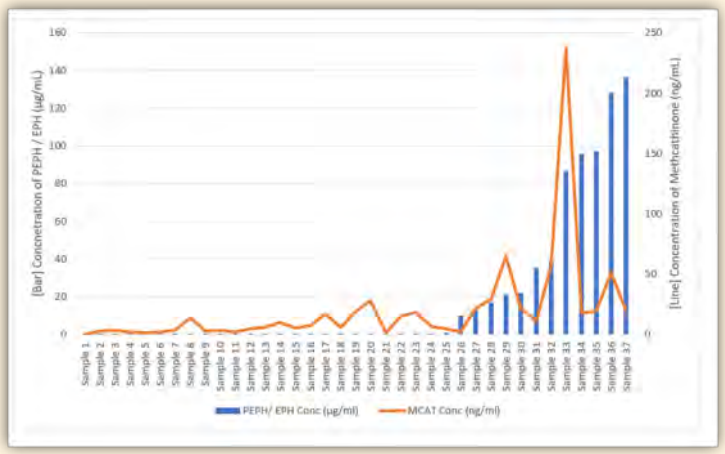


Figure 3. Concentrations of pseudoephedrine / ephedrine (bar) and methcathinone (line) detected in 37 suspected drug abusers' urine samples from routine forensic casework.

Higher amounts of methcathinone were found as compared to the volunteers' urine samples with 17 out of the 37 routine urine samples detected methcathinone at greater than 10 ng/mL. The highest amount of methcathinone was 238 ng/mL in Sample 33 which contained 86.8 µg/mL of pseudoephedrine or ephedrine *(not differentiated by analytical method used)*. In contrast, less than 2 ng/mL of methcathinone was detected in Volunteer 1's urine sample which has similar amount of pseudoephedrine (82.3 to 85.3 µg/mL). This could likely be attributed to the individual's metabolism, or that the subject of Sample 33 could have consumed ephedrine, possibly contributing to a higher conversion to methcathinone, as suggested in Figure 1.

Out of these 37 routine urine samples, 12 of them containing pseudoephedrine or ephedrine at greater than 10 µg/mL showed the concentration of methcathinone ranged from 1.92 to 239 ng/ml in the urine. The higher amounts of methcathinone detected in the routine urine samples (as compared to the spiked samples) seemed to suggest that methcathinone could be produced mainly from the metabolism of ephedrine or pseudoephedrine in the body.

While the mechanism that gives rise to this artefact in the testing procedure is unknown, it could be possible that during the process, oxidation could have occurred in the hydroxyl group at the C2 position (for ephedrine and pseudoephedrine), resulted in the formation of the keto group [1]. It was also reported in the same literature that ephedrine or pseudoephedrine are metabolic products of methcathinone and there are no studies to support that the reverse could take place metabolically. However, it is possible that this metabolic process could be reversible, nonetheless, produce a low level of methcathinone in the presence of ephedrine or pseudoephedrine in the body. It has been reported the detection of cathinone in equine after administration of norephedrine and a mechanism that generates a geminal diol as an intermediate has been postulated for the conversion of norephedrine (or norpseudoephedrine) to cathinone *in vivo* [2]. This conversion is catalyzed by dopamine-b-hydroxylase as shown in Figure 4(a). Hydroxylation of the b-C–H bond generated a geminal diol as the intermediate product, which exists in solution predominantly as the keto form [2]. Based on the similarity in structures between norephedrine–cathinone and ephedrine/pseudoephedrine–methcathinone, respectively, we postulate that the similar or same enzyme, dopamine b-hydroxylase could catalyze the conversion of ephedrine/ pseudoephedrine to methcathinone in the human body using the same mechanism, as shown in Figure 4(b).

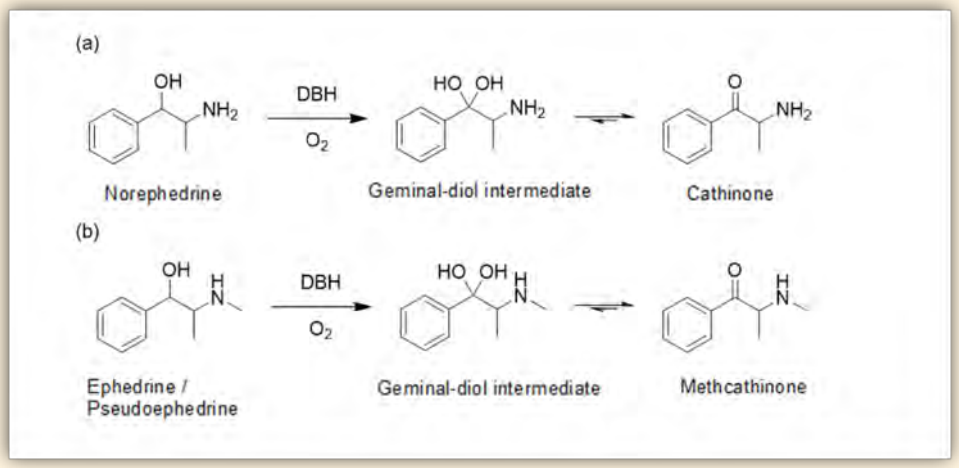


Figure 4: Proposed mechanism showing the formation of (a) cathinone from norephedrine [2] and (b) methcathinone from pseudoephedrine, catalyzed by dopamine-ß-hydroxylase (DBH).

A more recent publication [3] also supported the likelihood of metabolic formation of cathinone involving cathine (norpseudoephedrine) oxidation by dopamine b-hydroxylase via a germinal diol as an intermediate. Self-administration of pseudoephedrine yielded the detection of cathine, norephedrine and low levels of cathinone in the urine *(note: methcathinone was not tested by the authors)*. The authors concluded that the detection of cathinone in urine does not necessarily imply the consumption of khat, cathinone, or an illicit drug that metabolized to cathinone [3].

Our study of the routine urine samples which showed the presence of methcathinone *(no cathinone was detected in the samples)* suggested the possible conversion of ephedrine/pseudoephedrine to methcathinone via the same mechanism as depicted in Figure 4(b). The controlled administration study also demonstrated that the detection of trace amounts of methcathinone in the urine is not necessarily due to the consumption of illicit methcathinone.

Conclusion

From the spiked samples study, it was observed high concentrations of ephedrine and pseudoephedrine in water and urine could give rise to trace amounts of methcathinone during the sample preparation process. Results from the volunteers' urine samples after consumption of pharmaceutical tablets containing pseudoephedrine and routine urine samples containing high concentrations of pseudoephedrine or ephedrine showed the presence of methcathinone at low levels. This study has demonstrated that the detection of low levels of methcathinone in the urine samples could be contributed from both the sample preparation process and the metabolism of pseudoephedrine or ephedrine in the human body. In order to prevent any misinterpretation, the experimental observations in this study should be considered, especially in the presence of high concentrations of pseudoephedrine or ephedrine, before reporting methcathinone in routine urine tests.

References

[1] *Paul BD, Cole KA. Cathinone (khat) and methcathinone (cat) in urine specimens: a gas chromatographic-massspectrometric detection procedure. J Anal Toxicol. 2001; 25:525–530*

[2] *Yi R, Zhao S, Lam G, et al.. Detection and elimination profile of cathinone in equine after norephedrine (Propalin®) administration using a validated liquid chromatography tandem mass spectrometry method. Anal Bioanal Chem. 2013; 405 (30):9711-9722.*

[3] *Schwelm HM, Grumann C, Auwärter V, et al. Application of a chiral high performance liquid chromatography-tandem mass spectrometry method for the determination of 13 related amphetamine-type stimulants to forensic samples: Interpretative hypotheses. Drug Test Anal. 2020; 12:1354–1365.*

Artificial penile nodules as information for Identification of Unidentified Skeletal Remains: Case Reports

Ms Nattida Srinak
Central Institute of Forensic Science, Ministry of Justice, Bangkok, Thailand
Email: pairnattida@gmail.com

Introduction

The identification process implicates the comparison of information obtained by someone who knows the person (antemortem data, AMD) with the scientific information obtained by forensic experts during the examination of human remains (postmortem data, PMD). Skeleton Analysis Section (SAS) at the Central Institute of Forensic Science (CIFS), Thailand identifies Unidentified Skeletal Remains (USR) to provide a biological profile and the postmortem data of the USR, including the cause of death, pathologies, anomalies, surgical implants, and foreign objects. The Discovery of foreign objects is useful information because it is unique information. The foreign objects are additional evidence to support the identification to increase the possibility of true matches such as a comparison of antemortem and postmortem data [1]. This information can enhance the identification process.

One of the foreign objects that are often found in males around the male pelvis bone is a rounded shape bead. A bead is an object for Artificial Penile Nodules (APNs), which term is called “fang muk” in Thailand. The object is implanted in the subcutis of the penis, commonly in the prepuce or the dorsum of the penile shaft (Figure 1) [2]. Some men believe that penile implants increase the pleasure of sexual partners [3]. The bead is made from different materials such as plastic, metal, glass, ivory, silicon, wood, marble, and pearls. There are various styles of implant (e.g., capsules, oval, pyramids, and rectangular) [4]

The implantation of penile objects has been adopted by people with low socio-economic levels and is commonly carried out in prisons [2]. In Thailand, 61% of young amphetamine users were commonly implanted with glass beads [5]. These groups tend to be homeless or missing persons and subsequently become part of the unidentified human remains [6]. Penile foreign body implantation has been reported in several ethnic and social groups, mainly of Asian origin [7].

The forensic identification of human remains must take a holistic approach: all information that could help identify a body or set of human remains should be considered. So, these case reports aim to emphasize the importance of the APNs in unidentified human remains in individual identification.

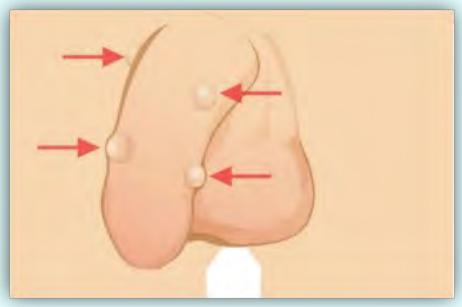


Figure 1. A diagram shows the artificial penile nodules along the penile shaft, representing the penile bead implant (red arrow) [7].

Case Reports

Case 1

In March 2018, the USR was sent to the SAS laboratory to identify the biological profile. The result showed that the remains was an Asian male with an age range between 40-50 years old. The stature was between 159.15 -169.27 centimeters. A small, rounded shape glass bead with opaque white color was found around the pelvis bone. (fig. 2-3). The diameter of the bead was 7.50 millimeters.

Figure 2. Skeleton, from USR of case 1, laid out in anatomical position.

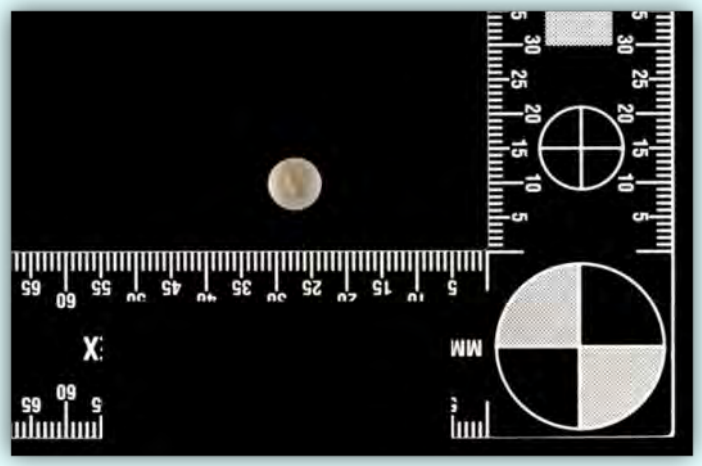


Figure 3. A white opaque penile bead found in case 1.

Case 2

In December 2022, a decomposed body was sent to the SAS laboratory. After the soft tissue removal process was done, the bones were identified. The result showed that the remains was Asian male with an age range between 45-50 years old. The stature was between 162.69-172.81 centimeters. A small, rounded shape glass bead with opaque white color was found around the pelvis bone. (Figure 4-5). The diameter of the bead was 7.90 millimeters.

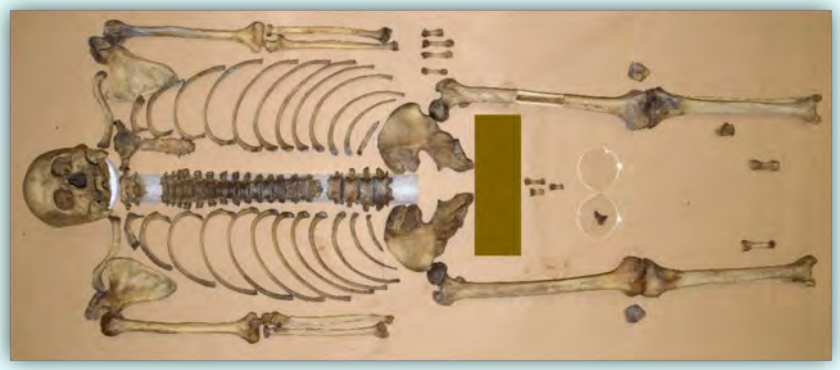


Figure 4. Skeleton, from USR of case 2, laid out in anatomical position.

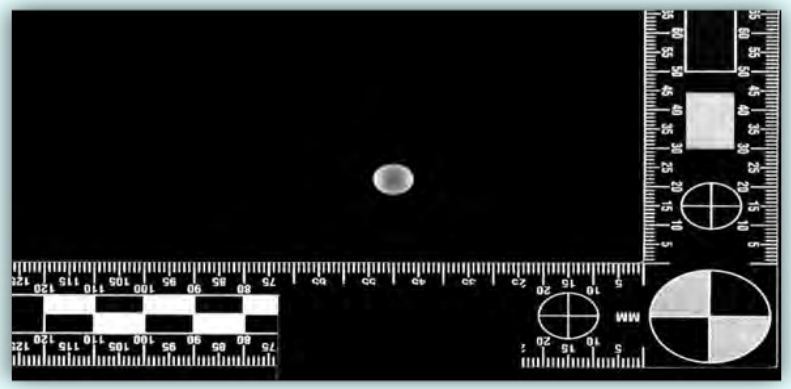


Figure 5. A white opaque penile bead found in case 2

In both cases, FT-IR spectrometer and X-Ray fluorescence spectrometer were used to analyze the beads, the methods revealed that the beads were glass, and the specific gravity was 2.45.

Discussion

The discovery of the APNs in unidentified human remains is novelty information. From the report, both cases were male, and the beads were found around the pelvis that was consistent with the location to implant; the penis. the APNs were a common finding in Asian countries especially Southeast Asia such as Indonesia Singapore, Malaysia, Vietnam, and Cambodia that corresponding with the ancestry from these reports [3]. The age range of USR that is related to the implantation of penile objects is elderly. This phenomenon may be popular in the past because of low socio-economic levels and less access to education of people in Thailand which was different from many previous studies [8-9].

Conclusion

The discovery of the APNs in USR by skeleton analysis method can provide additional information to assist in personal identification. These reports show the picture and information of the APNs involved with the remains that may be useful in future studies.

References

[1] Coupland R, Tidball-Binz M. Missing people, DNA analysis and identification of human remains.

[2] Jalink M, Kramp KH, Baktawar S, Jewbali A. Skin necrosis after self-removal of an artificial penile nodule in a Surinamese man. Case Reports. 2016 Jun 28;2016:bcr2015214042.

[3] Fischer N, Hauser S, Brede O, Fisang C, Müller S. Implantation of artificial penile nodules—a review of literature. The journal of sexual medicine. 2010 Nov;7(11):3565-71.

[4] Bjekić M. Artificial penile nodules: a case series of three patients. Serbian Journal of Dermatology and Venereology. 2013;5(4):165-70.

[5] Thomson N, Sutcliffe CG, Sirirojn B, Sintupat K, Aramrattana A, Samuels A, Celentano DD. Penile modification in young Thai men: risk environments, procedures and widespread implications for HIV and sexually transmitted infections. Sexually transmitted infections. 2008 Jun 1;84(3):195-7.

[6] Kimmerle EH, Falsetti A, Ross AH. Immigrants, undocumented workers, runaways, transients and the homeless: Towards contextual identification among unidentified decedents. Forensic Science Policy and Management. 2010 Mar 16;1(4):178-86.

[7] Levy G, Mercer D, Amosi D, Arad E. Self-implanted artificial nodules: a computed tomography mimic of penile pathology. Acta Radiologica. 2008 Mar;49(2):236-8.

[8] กลุ่มตรวจวิเคราะห์กระดูก.Artificial Penile Nodules.กระดูกผูกเรื่องราว.E-book[Internet].2022 cited 2023 Feb 15];2022:12-13. Available from: https://thaimissing.go.th/article/9

[9] Wilcher G. Artificial Penile Nodules–A Forensic Pathosociology Perspective. Medicine, science and the law. 2006 Oct;46(4):349-56.

The Role of Forensic Anthropology in Thailand for personal identification of unidentified human remains: A case study

Ms Nattida Srinak
Central Institute of Forensic Science, Ministry of Justice, Bangkok, Thailand
Email: pairnattida@gmail.com

Introduction

Forensic anthropology (FA) is the subdiscipline of physical/biological anthropology that applies their knowledge of human skeleton variation to help law enforcement identify unidentified human remains (UHR) and, if possible, provide information about the circumstances surrounding a death. Forensic anthropologists employ the principle of skeletal growth, development, degeneration, and variation to establish a biological profile about an individual, such as sex, age, ancestry, and stature, including biomechanics and bone healing, to evaluate skeletal trauma. When other physical features of the remains were distorted by decomposition or injury, the FA has been used to identify the biological profile of the remains [1].

One of the missions of the Skeleton Analysis Section, Central Institute of Forensic Science, Thailand, is to identify the UHR (skeletal form) for providing the biological profile. Since 2002, we had over 2,000 UHR cases. So, to realize the importance of the FA for personal identification-The case study was explained.

Case study

On 21 June 2021, the UHR was transferred to the FA laboratory to perform the identification person. The remains were advanced decomposition. Face and other physical features were distorted by decomposition. The remains could not provide any information.

Method

The remains were cleaned for subsequent analysis. After that, they were sorted and sided in anatomical positions (Figure 1). General information, including the number and identity of the bones, was recorded [2]. In the case, none of the skeletal elements were redundant, and the remains were consistent with a single individual—subsequently, analysis biological profile and information about the remains.

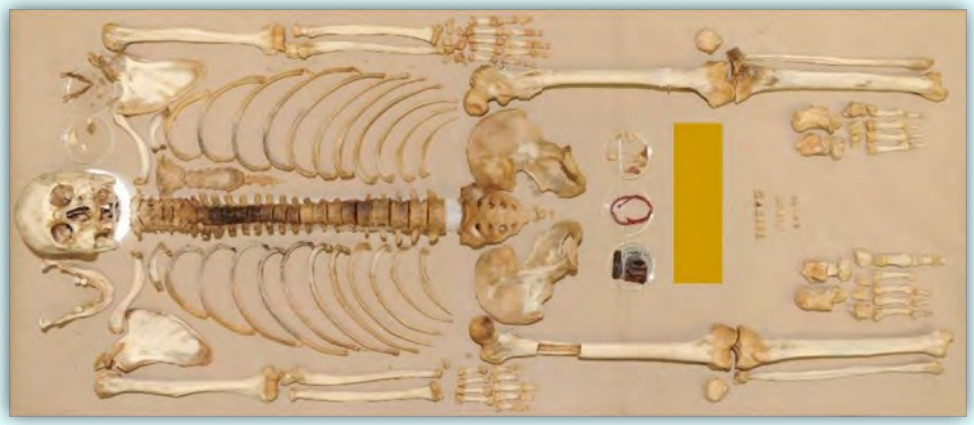


Figure 1. Anatomical display of a skeleton for inventory and analysis.

Result and discussion

Biological profiles

Pelvis and skull were used to determine sex by the non-metric trait method (Figure 2a, b) because the pelvis provides the most accurate results in the determination of sex (95% accuracy), followed by the skull (92% accuracy) [3]. Sex can be distinguished by observing size and rugosity. Male skull is more robust, especially at the muscle insertion sites. Architectural differences also distinguish the sexes; there are several shape characteristics of the female pelvis related to childbirth. Thus, these structures are wider than those of males of comparable size [4]. In the case, all these characteristics were consistent with a male individual.

Figure 2. Pelvis (a) and skull (b) of the unidentified human remains.

The remains were adult because the dentition fully emerged, and growth had ceased—the method for determining age at death of an adult base on the deteriorating skeleton. The age can be determined by comparing the status of these structures in skeletons with accepted schedules of these changes [4]. Four osteological features alter during adulthood [5]: pubic symphysis of the pubic bones, auricular surfaces of the ilium, sternal rib ends, and maxillary sutures (Figure 3a-d) were used. From the analysis result, all these methods estimated the age at death 50-64 years old.

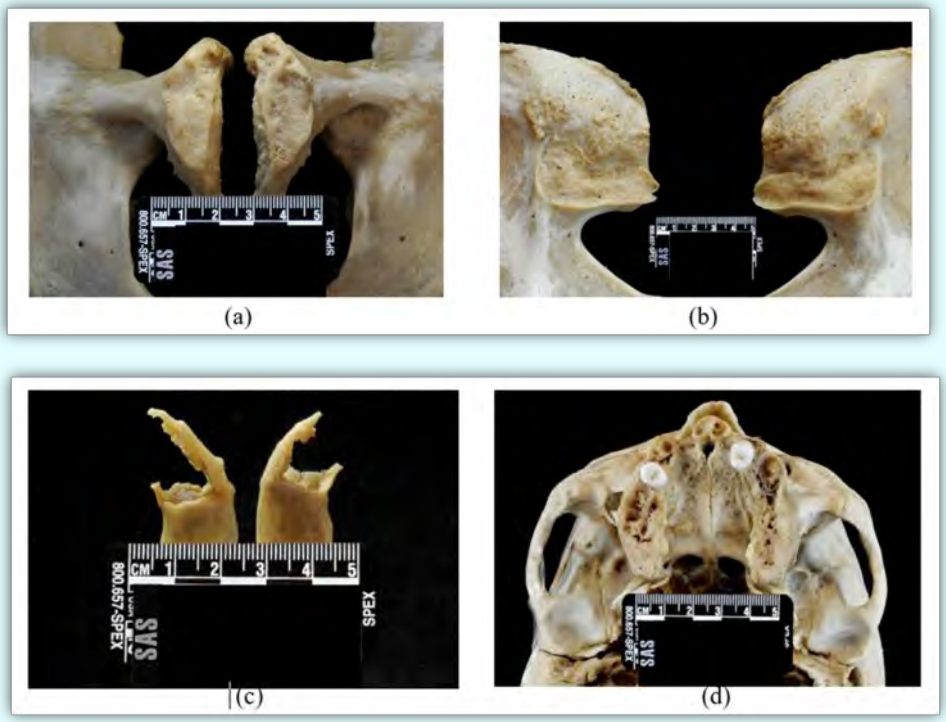


Figure 3. pubic symphysis (a) auricular surface (b) sternal end of 4th rib (c) and maxillary suture (d).

Many skull characteristics, such as nasal opening, eye orbits, and palatal shape, have been used for ancestry. In this case, all these features supported the estimate as Asian. The right femur's maximum length was measured using linear regression to estimate stature. The formulae generated from a northern Thai population were used [6]. In the case, the estimated stature of the remains was 170.59-180.71 cm.

Trauma

Antemortem trauma

Antemortem trauma refers to trauma that occurred before death that shows signs of healing and remodeling bone. In this case, a surgical implant, intramedullary nailing, was found on the shaft of the left femur (Figure 4). The finding indicated that the remains had broken the left femur before death.

Figure 4. Intramedullary nailing on the shaft of the left femur.

Perimortem trauma

The cranium was fragmentary, so several elements were reconstructed (Figure 5a, b). Cranium showed evidence of blunt force trauma and exhibited evidence of perimortem trauma. Perimortem trauma occurs at/or around the time of death.

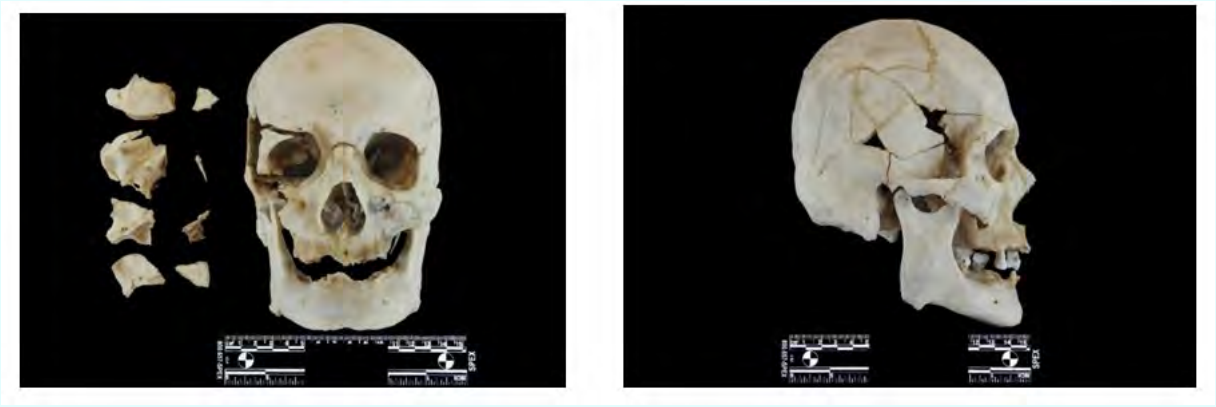


Figure 5. Cranium before reconstruction (a) and after reconstruction (b).

Comparative data

Antemortem data from the suspect's missing person compared with data from skeleton analysis as follows:

Biological profiles	Data from suspected missing person	Data from skeleton analysis	Consistent
Sex	Male	Male	✓
Age	55 years old	50-64 years old	✓
Ancestry	Asian	Asian	✓
Stature	≈180.00 cm.	170.59-180.71 cm.	✓

Table 1. Comparation data from suspected missing person and skeleton analysis.

From the results found that both data were consistent, so the officer confirm person by DNA testing; the DNA testing result showed the same person result.

Conclusion

The FA is one of the practical methods to identify unknown dead bodies. It can provide important data for identifying people. The data from the anthropological method can be compared with missing person data to exclude and scope the data between UHR and missing persons. In addition, trauma was also helpful for a pathologist to examine the cause of death; other methods could not provide this data. So, FA can utilize for establishing biological profiles and interpreting trauma to examine the cause of death. However, there still needs to be a method to confirm a person, such as DNA testing or forensic odontology.

References

[1] Langley NR, Tersigni-Tarrant MA, editors. Forensic anthropology: a comprehensive introduction. CRC press; 2017 Feb 24.

[2] Steadman DW, editor. Hard evidence: case studies in forensic anthropology. Routledge; 2015 Aug 7.

[3] Kanchan T, Krishan K. Personal identification in forensic examinations. Anthropol. 2013;2(1):114.

[4] Byers SN. Introduction to forensic anthropology. Taylor & Francis; 2016 Sep 19.

[5] Buikstra JE. Standards for data collection from human skeletal remains. Arkansas archaeological survey research series. 1994;44.

[6] Mahakkanukrauh P, Khanpetch P, Prasitwattanseree S, Vichairat K, Case DT. Stature estimation from long bone lengths in a Thai population. Forensic science international. 2011 Jul 15;210(1-3):279-e1.

Case Study

Application of Gas Chromatography Mass Spectrometry Technique for the Identification of Adulterants in Seized Captagon Tablets

Dr.Vanitha Kunalan, Ms.Siti Zahara Abu, Ms.Norhaya Ramli & Ms.Julaineh Jumin*
Nacrotics Division, Forensic Science Analysis Centre, Department of Chemistry Malaysia, Malaysia
Email: vanitha@kimia.gov.my

Abstract

Amphetamine type stimulants (ATS) are the second most popular illegal drugs used worldwide, after cannabis. The production of ATS has increased across the world, including the Middle East. Fenethylline, commonly known by its street name 'Captagon' was seized for the first time in Malaysia in 2021. Samples from two big seizures were analysed by means of gas chromatography-mass spectrometry (GC-MS). The analysis indicated the presence of amphetamine together with several adulterants and diluents. Based on GC-MS analytical data, comparison was made between the samples from the two different seizures to determine if similarities could be observed to make inferences on the origin of these seizures. In this article, the findings from both case studies will be presented.

Introduction

ATS such as amphetamine, methamphetamine, fenethylline, methylphenidate, and dextroamphetamine are synthetic drugs belonging to the stimulant class that excite the central nervous system (CNS) to produce effects similar to adrenaline[1,2]. ATS are the second most popular illicit drugs used worldwide, after cannabis[3,4].

Fenethylline, 1,3-dimethyl-7-[2-(1-phenylpropan-2-ylamino)ethyl]purine-2,6-dione in Figure 1, is a theophylline derivative of amphetamine having effects similar to those of the amphetamine-type stimulant (ATS)[5]. During the 1960s and 1970s, fenethylline was used for its anti-depressant properties. It was formerly available as a medicinal product in the form of white-coloured tablets stamped with an imprint of two half-moons, marketed under the brand name 'Captagon' (Figure 2).

The illicit production of fenethylline in illegal laboratories was recorded nearly a decade ago by the International Narcotics Control Board and the Interpol–although legal production of fenethylline was banned in many countries in 1986 in view of its inclusion in Schedule II of the United Nation (UN) Convention on Psychotropic Substances 1971[6,7].

Unlike plant-based drugs such as heroin and cocaine, ATS manufacture is relatively easy, making use of common household chemicals and solvents that are easily available from commercial sources. In addition, methods of production for such drugs are also readily available on the Internet. This has contributed to the widespread illicit production of these synthetic drugs among many populations with high incidence of adulteration. Like many other illicit drugs, ATS are rarely sold or used in their pure state. They are often extensively adulterated with a variety of substances, in order to increase the apparent amount of the drug, thereby increasing the dealer's profit. Identification of such adulterants and diluents are important in view of the health risks and the possibility of intoxication.

The manufacture of fenethylline (active ingredient in Captagon tablets) often make use of amphetamine and theophylline as the starting materials. As such, improper street manufacture of such tablets may result in the high yield of these 2 compounds in the final product and sometimes with the absence of fenethylline, itself.[6,8]

This article presents the methodology used to identify the psychoactive substances and its co-existing adulterants and diluents in two Captagon tablets seizures found in Malaysia using GC-MS.

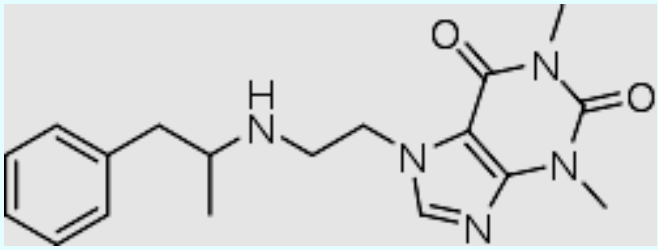


Figure 1. Chemical structure of fenethylline.

Figure 2. Captagon tablets[9]

Analysis

The tablets from both seizures were each pulverised for analysis. Approximately 50 mg of the pulverised tablet was placed in a 5 mL volumetric flask with 5 mL of methanol added. The sample solution was sonicated for 10 minutes and left to stand for another 10 minutes. The clear methanol extract was then analysed by GC-MS.

Figure 3. Images of tablets hidden inside castor wheels from the seizures found in Malaysia.

Gas Chromatography/Mass Spectrometry (GC-MS)

An Agilent 6890N GC equipped with an Agilent 5973 quadrupole mass-selective detector was used with the following method parameters:

- Column: HP-5: 30 m x 0.25 mm i.d., 0.25 mm film thickness
- Carrier gas: Helium
- Injection mode: Split mode (1:50)
- Injection Volume: 1.0 µL
- Oven Temperature: 150°C for 1 minute, 30°C/minute to 270°C and hold for 3.5 minute
- Column flow rate: 0.9 mL per minute
- Injector temperature: 250°C
- Detector temperature: 280°C
- Run Time: 8.5 minute

Results And Discussion

Gas chromatography coupled with mass spectrometry is regarded as the "gold standard" for drug confirmation in forensic analysis. The GC chromatograms of the seized Captagon tablets from both case studies were shown in Figure 4-5. In both seizures, the absence of fenethylline was observed while the presence of amphetamine was detected. In addition, other adulterants and diluents were also found. A list of the compounds detected with their corresponding retention time and mass ions were given in Table 1 while the mass spectra (electron ionization mode) for 3 of the compounds were shown in Figure 6-8.

The absence of fenethylline, the active ingredient found in Captagon tablets, coupled with the presence of amphetamine together with other adulterants and diluents suggested that the tablets recovered from these two seizures were clandestinely produced. Through these 2 case studies, it was demonstrated that GC-MS is a useful technique that is able to differentiate the psychoactive drug fenethylline/amphetamine from its adulterants and diluents found in Captagon tablets. The differences observed in the GC profiles of these 2 tablet seizures also suggested the possibility of a different sample origin. Amphetamine is currently listed in Schedule 1 of the Malaysian Dangerous Drugs Act (DDA) 1952.

No	Compound	Retention Time (min)	Major fragment ions m/z
1	Amphetamine	1.90	44, 91, 65, 120, 51, 134
2	Acetaminophen	3.86	109, 151, 80, 43, 53
3	Caffeine	4.45	194, 109, 67, 55, 82, 165, 42
4	Diphenhydramine	4.56	58, 73, 165, 152, 45
5	Diphenylisopropyl amine	4.66	162, 91, 119, 44, 238, 252
6	Unknown	4.91	152, 136, 91, 123, 79, 65, 41
7	Theophylline	4.93	180, 95, 68, 53, 41, 151, 123

Table 1. A list of the compounds found in seized Captagon tablets.

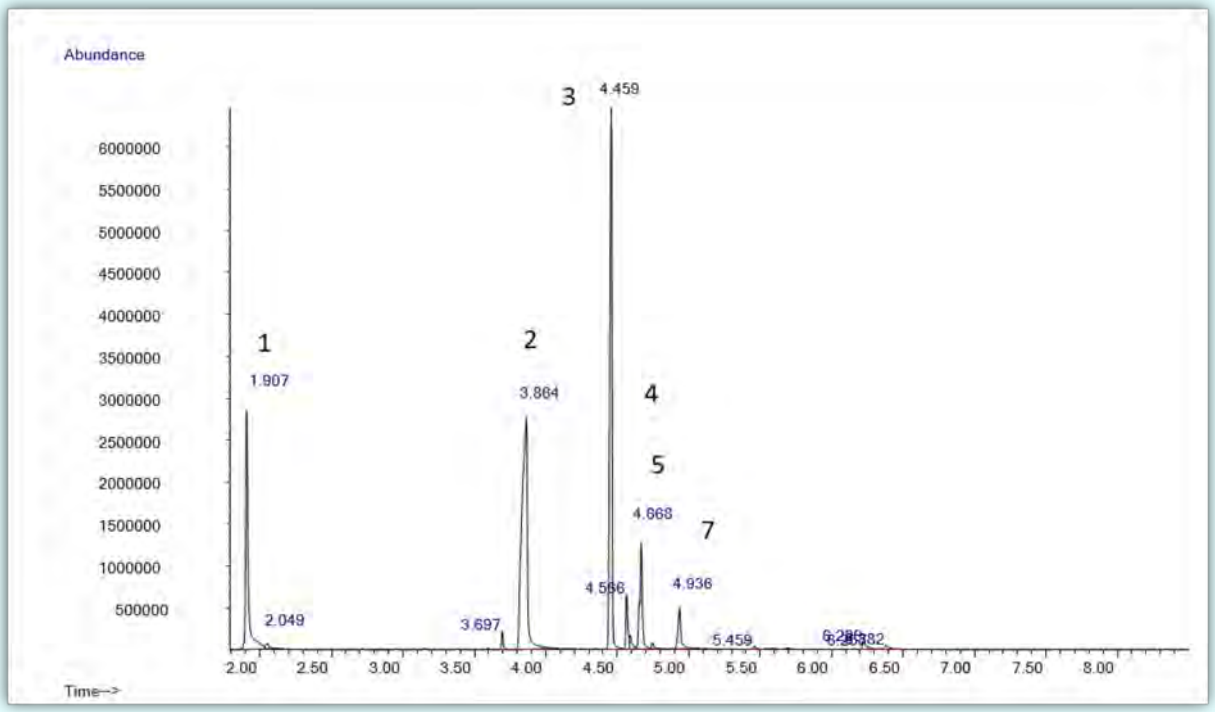


Figure 4. GC Chromatogram of tablet from case study 1.

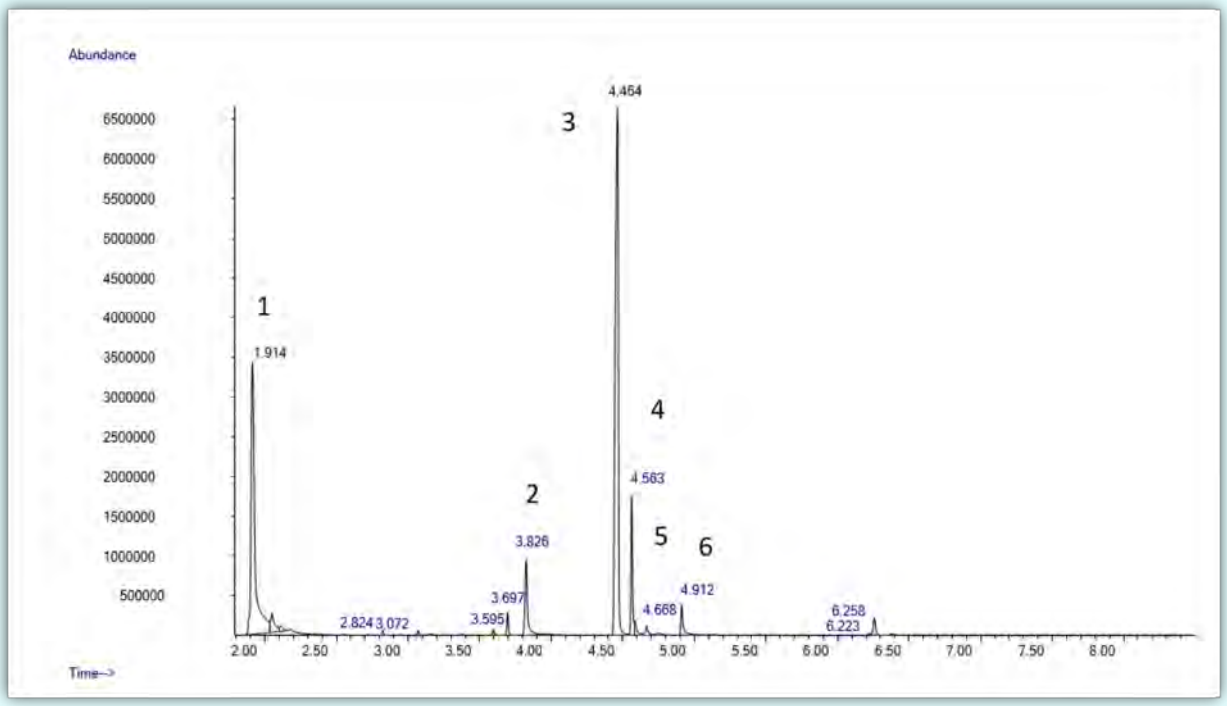


Figure 5. GC Chromatogram of tablet from case study 2.

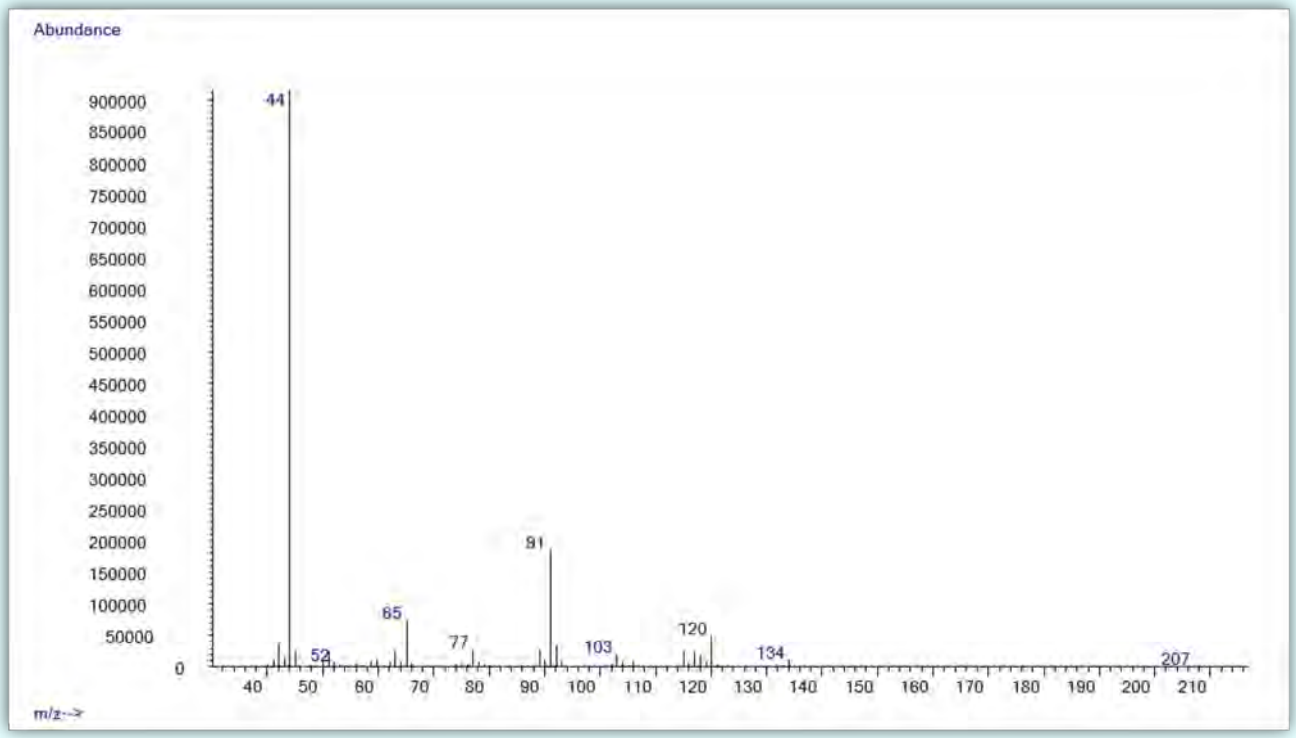


Figure 6. EI-MS spectrum of amphetamine.

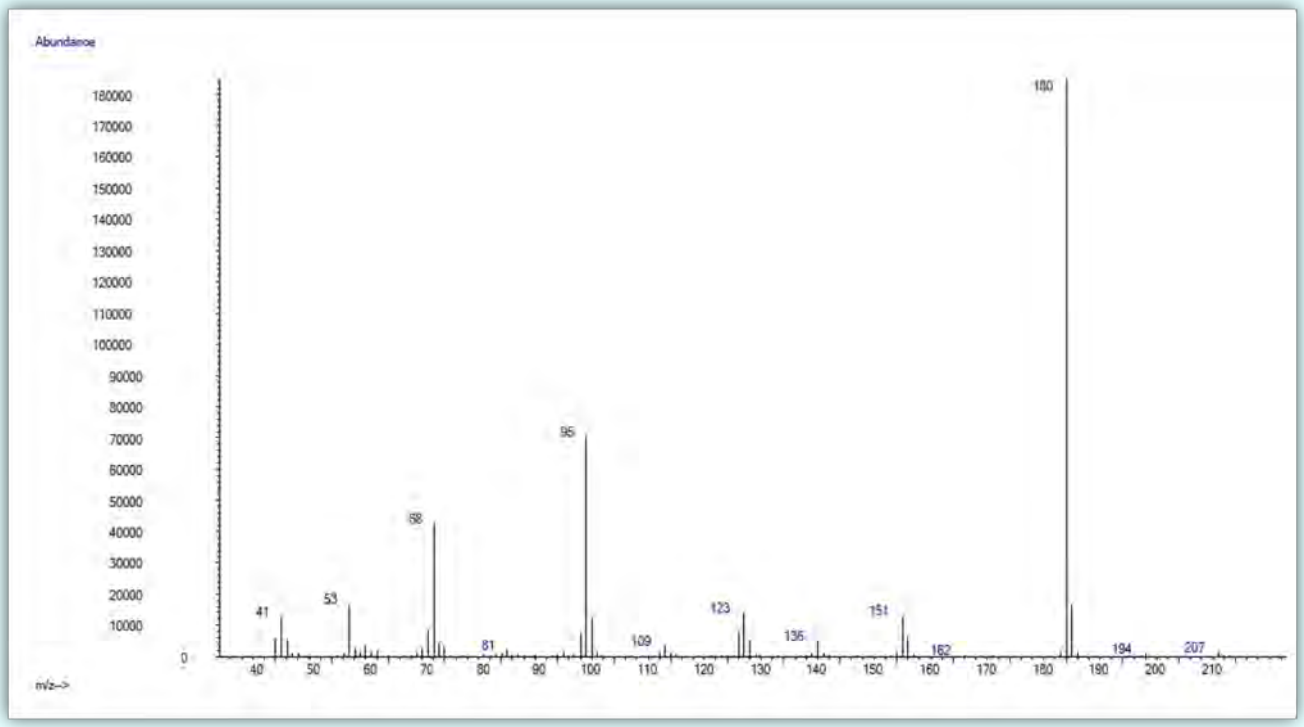


Figure 7. EI-MS spectrum of theophylline.

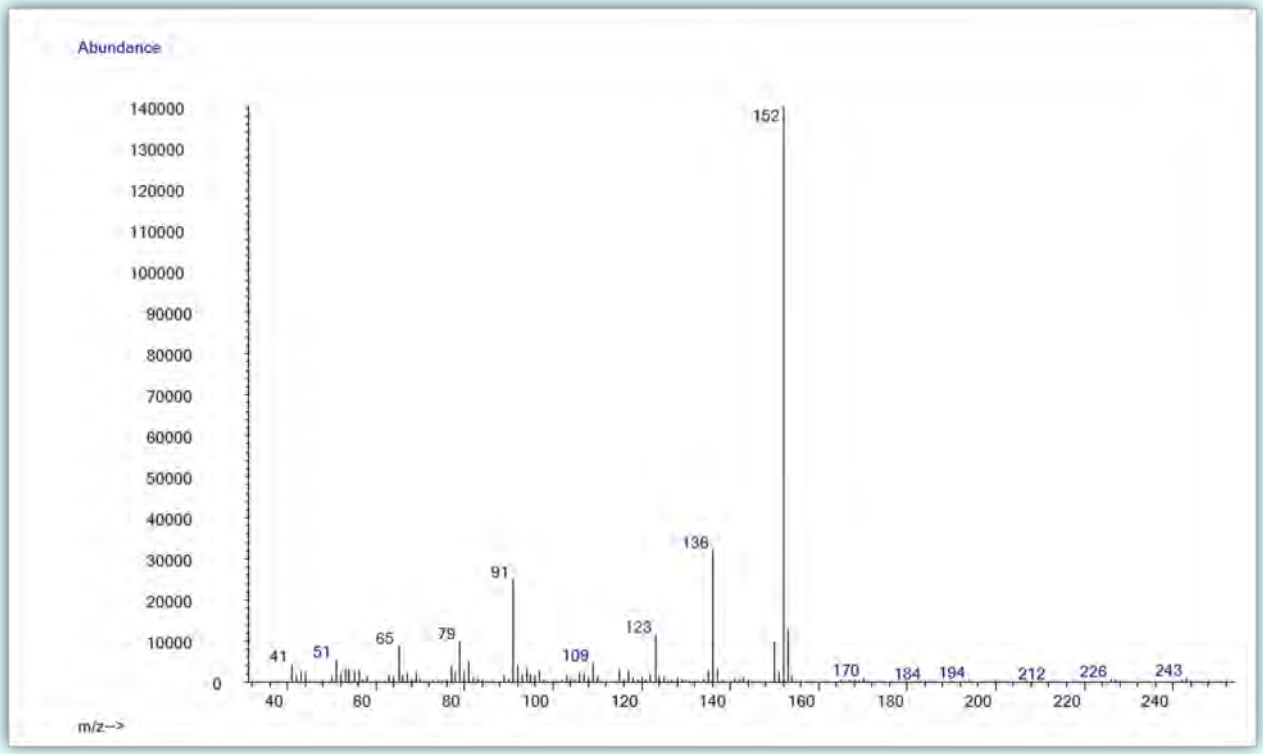


Figure 8. EI-MS spectrum of unknown.

Conclusion

The 2 case studies presented had demonstrated the usefulness of GC-MS in confirming the identity of the adulterants and diluents present in seized Captagon tablets. Based on GC-MS analytical data, comparisons could be made between the samples from different seizures to make inferences with regards to the commonality of their origin. The absence of fenethylline and the presence of amphetamine and theophylline together with other adulterants and diluents in the two case studies presented suggested that the 2 Captagon tablets seizures are clandestinely produced. The differences observed in their GC profiles further suggested the possibility of these 2 seizures coming from a different origin.

References

[1] *Shadloo, B.; Amin-Esmaeili, M.; Haft-Baradaran, M.; Noroozi, A.; Ghorban-Jahromi, R.; Rahimi-Movaghar, A. Use of Amphetamine-Type Stimulants in the Islamic Republic Of Iran, 2004–2015: A Review. East. Mediterr. Health J. 2017, 23, 245–256.*

[2] *WHO WPRO., 2017. Patterns and consequences of the use of amphetamine-type stimulants (ATS). World Health Organization.*

[3] *UNODC., 2020. World Drug Report. [online] Vienna: United Nations Office on Drugs and Crime.*

[4] *UNODC., 2021. World Drug Report. [online] Vienna: United Nations Office on Drugs and Crime.*

[5] *Sweetman, S. C. Martindale: the complete drug reference; The Pharmaceutical Press: London, 2002; p. 1509.*

[6] *Haya I.Aljohar et al. Gas chromatography tandem mass spectrometry for the screening of adulterants in seized captagon™ tablets.*

[7] *Ali Zahid A Alshehri et al GC-MS analysis of Adulterants in Captagon Tablet.*

[8] *Maria Katselou et al Fenethylline (Captagon) Abuse – Local Problems from an Old Drug Become Universal.*

[9] *https://www.publish0x.com/world-international-news-group/italian-police-seizes-14-tonnes-of-counterfeit-captagon-pill-xwnvegg*

AFSN Member Institutes

Country/Region	*No.*	*Name of Member Institute (as at June 2023)*
Bangladesh	1	National Forensic DNA Profiling Laboratory
Brunei Darussalam	2	Department of Scientific Services
India	3	Centre for DNA Fingerprinting and Diagnostics
	4	Directorate of Forensic Science, Himachal Pradesh
Indonesia	5	Centre of Indonesian Automated Fingerprint Identification System of the Indonesian National Police
	6	Department of Police Medicine of the Indonesian National Police
	7	Eijkman Institute for Molecular Biology
	8	Forensic Laboratory Centre of Indonesian National Police Headquarters
	9	Indonesian Association of Forensic Pathologist
	10	Laboratory of National Narcotics Board
	11	Master Program of Forensic Science, Postgraduate School, Universitas Airlangga
Lao PDR	12	Food and Drug Quality Control Center
Malaysia	13	CyberSecurity Malaysia
	14	Department of Chemistry
	15	Malaysian Communications and Multimedia Commission
	16	National Institute of Forensic Medicine, Hospital Kuala Lumpur
	17	Royal Malaysia Police Forensic Laboratory
Mongolia	18	Mongolian National Institute of Forensic Science
People's Republic of China	19	Beijing Forensic Science Institute
	20	Criminal Investigation School, Southwest University of Political Science and Law (SWUPL)
	21	Forensic Science Center of Guangdong Provincial Public Security Department
	22	Forensic Science Division, Department of Fujian Provincial Public Security
	23	Gansu University of Political Science and Law, Key Laboratory of Evidence Science Techniques Research and Application
	24	Guangzhou Forensic Science Institute
	25	Institute of Forensic Science, Ministry of Public Security
	26	Institute of Forensic Science, Dezhou Public Security Bureau
	27	Institute of Forensic Science, Hangzhou Public Security Department
	28	Institute of Forensic Science, Shandong Public Security Department
	29	Institute of Forensic Science, Suzhou Public Security Bureau
	30	Institute of Forensic Science, Tianjin Public Security Bureau
	31	The Institute of Evidence Law and Forensic Science, China University of Political Science and Law
	32	Forensic Science Division of the Government Laboratory, Hong Kong Special Administrative Region
	33	Forensic Science Department of Judiciary Police, Macau Special Administrative Region
Philippines	34	Laboratory Service, Philippine Drug Enforcement Agency
	35	National Bureau of Investigation
	36	National Reference Laboratory for Environmental and Occupational Health, Toxicology and Micronutrient Assay, East Avenue Medical Center, Department of Health
	37	Natural Sciences Research Institute, University of the Philippines Diliman Quezon City

Country/Region	*No.*	*Name of Member Institute (as at June 2023)*
Philippines	38	Philippine National Police
Republic of Kazakhstan	39	Forensic Examinations Centre of the Ministry of Justice of the Republic of Kazakhstan
Republic of Korea	40	Daejeon Health Institute of Technology, Daejeon Health Sciences University
	41	Department of Forensic Sciences, Sungkyunkwan University
	42	Graduate School of Forensic Science, Soon Chun Hyang University
	43	Institute of Forensic and Anthropological Science
	44	Korea Coast Guard Research Institute
	45	National Digital Forensic Center of Supreme Prosecutors' Office
	46	National Forensic Service
	47	Scientific Investigation Center of Korean National Police Agency
	48	Scientific Investigation Laboratory, Ministry of National Defense
	49	SJS Institute of Forensic Science & Medicine
Republic of Uzbekistan	50	Republican Scientific and Practical Center of Forensic Medical Examination, Ministry of Health, Republic of Uzbekistan
	51	Republican Center for Forensic Examination under the Ministry of Justice, Republic of Uzbekistan
Singapore	52	Corrupt Practices Investigation Bureau
	53	Health Sciences Authority
	54	Ministry of Home Affairs
Sri Lanka	55	Government Analyst's Department
	56	National Dangerous Drugs Control Board
Thailand	57	Central Institute of Forensic Science
	58	Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University
	59	Department of Forensic Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University
	60	Division of Forensic Medicine, Thammasat University Hospital
	61	Department of Medical Sciences
	62	Faculty of Medicine, Chiang Mai University
	63	Human Genetics Unit, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital
	64	Institute of Forensic Medicine, Police General Hospital, The Royal Thai Police
	65	Narcotics Analysis and Technical Service Institute Office of Narcotics Control Board
The Republic of the Union of Myanmar	66	Defence Services Medical Research Centre
Timor-Leste	67	Polícia Científica de Investigação Criminal - Laboratório de Polícia Científica
Vietnam	68	Forensic Medicine Center of Ho Chi Minh City
	69	National Institute of Forensic Medicine
	70	Forensic Science Institute Vietnam