ForensicAsia
THE ASIAN FORENSIC SCIENCES NETWORK NEWSLETTER | ISSUE 9 | 2019

AFSN President's Address

Dear colleagues and friends,

The 10th AFSN Annual Meeting and Symposium, hosted in Beijing from 4 to 8 September 2018 by Institute of Forensic Science, Ministry of Public Security, China, had been a great success. As my home institute, IFS was very happy to host the meeting and treated every participant. Here I want to express my gratitude for every friend, it was the participation, support and cooperation from everyone that made the meeting amazing.

Nowadays, all countries in the world are interrelated and interdependent, and the destiny of the world is closely related to common interest. Peace, development, cooperation and win-win situation have become an irresistible historical trend. At the same time, we are facing the common challenges of terrorism, cybercrime, transnational organized crime and other global security issues. Forensic science, as a scientific and technological method to study and identify the nature of crimes and events, to discover, to expose, to prove and to prevent crimes, runs through all aspects of justice and is the key foundation of law enforcement. Cooperation in forensic science is a must and the best option. AFSN not only provides a good communication platform, but also promotes the overall quality of forensic science in Asia through technical exchanges and personnel training. It is expected that AFSN will provide better support for law enforcement cooperation and contribute more to the fight against transnational crime and the maintenance of regional and world security.

There are 56 member institutes from 17 countries as at 28 February 2019, 8 workgroups (DNA, Trace Evidence, Crime Scene Investigation, Illicit Drugs, Toxicology, Digital Forensic, Questioned Document and Fingerprint) and a Quality Assurance & Standards Committee. We must develop ourselves to answer the new challenges, work together, to construct, to share and to form consensus. We will try our best to create more opportunities and different ways to enhance collaboration among different forensic institutes in countries within or outside Asia.

I am also happy to announce that the 11th AFSN Annual Meeting and Symposium will take place in Ho Chi Minh City from 17 to 20 September 2019, hosted by Medical Forensic Center of Ho Chi Minh City, Vietnam. Although the Medical Forensic Center of Ho Chi Minh City is a new member who joined AFSN last September, the staff show strong willing to hold the 11th Annual Meeting. I want to take this opportunity to thank Dr Phan Van Hieu, the director of the Center, for his great enthusiasm and support to AFSN.

I will continue to work with all board members, and to play a more active role to achieve our goals, constructing AFSN to become an active and successful community in forensic science.

Thank you & Best Wishes!

Zhao Qiming
AFSN President,
Director-General,
Institute of Forensic Science,
Ministry of Public Security,
P. R. of China

Content

Editorial Committee

For enquiries, feedback or contribution of articles, please email to hsa_asg@hsa.gov.sg. For contribution of articles, please read the guidelines at www.asianforensic.net.

Editor's Address

Dear colleagues and members of AFSN,

As a result of the overwhelming response on the number of articles we received for the 8th Issue of *ForensicAsia* last year, we have to extend the publication of some of the articles to this 9th Issue.

In this Issue, we have a total of 5 technical articles and one case study, including, DNA, illicit drugs, toxicology, and general forensic science. We have received contributions on AFSN news on the birth of 2 new Workgroups, namely, Questioned Document and Digital Forensic. Member institutes are encouraged to join these two Workgroups. In addition, we also have one article on the development of ISO forensic standards in the international scene as well as introduction of five new member institutes which joined AFSN last year.

Once again, I would like to take this opportunity to thank all the authors who have contributed to the articles to this 9th Issue of *ForensicAsia.* I would also like to thank our editorial committee who spent their time in reviewing the articles, and our editorial assistants who put together all the articles and artwork of this new publication. In particular, I would take this opportunity to thank Dr Seah Lay Hong for her dedication and contribution to review many of the articles in the past issues. As she has left Department of Chemistry, Malaysia, and could no longer be our guest editor, we wish her all the best in her new job in the Universiti Sains Malaysia.

Happy reading!

Dr Lui Chi Pang
Editor

Upcoming Events

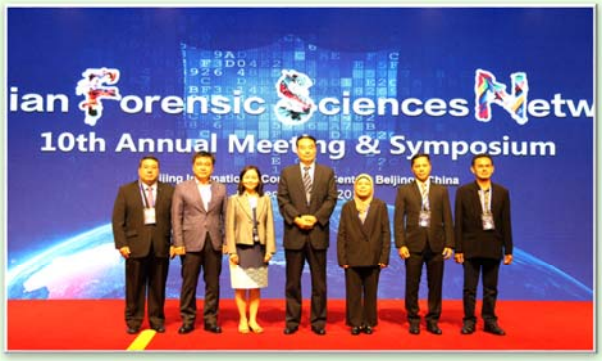
Date	Event Name
19 May – 23 May 2019	American Society of Crime Laboratory Directors (ASCLD) 46th Annual Symposium, St Louis, MO, United States of America
22 May - 24 May 2019	Forensic International Network for Explosives Investigation (FINEX) Conference, France
26 May – 31 May 2019	50th Annual Association of Firearm and Tool Mark Examiners (AFTE) Training Seminar, Nashville, TN, United States of America
3 Jun – 6 Jun 2019	Institute of Police Technology Management's (IPTM) Symposium on Traffic Safety, Orlando, Florida, United States of America
10 Jun – 14 Jun 2019	Inter/Micro 2019, Chicago, IL, United States of America
17 Jun – 21 Jun 2019	International Association of Bloodstain Pattern Analysts (IABPA) European Conference, Paris, France
18 Jun – 19 Jun 2019	Human Identification Solutions (HIDS) Conference, Kobe, Japan
11 Aug – 17 Aug 2019	104th International Association for Identification Conference, Reno, Nevada
2 Sep – 6 Sep 2019	The International Association of Forensic Toxicology (TIAFT) 57th Annual Meeting, Birmingham, United Kingdom
3 Sep – 7 Sep 2019	Clandestine Laboratory Investigating Chemists (CLIC) 29th Annual Meeting, Philadelphia, PA, United States of America
9 Sep – 13 Sep 2019	The 28th Congress of the International Society for Forensic Genetics, Prague, The Czech Republic
16 Sep – 19 Sep 2019	7th Forensic Isotope Ratio Mass Spectrometry Conference, San Michele all'Adige, Italy
17 Sep – 20 Sep 2019	11th AFSN Annual Meeting and Symposium, Ho Chi Minh City, Vietnam
23 Sep – 26 Sep 2019	30th International Symposium on Human Identification (ISHI), Palm Springs, CA, United States of America
8 Oct – 11 Oct 2019	18th Annual Association of Forensic Quality Assurance Managers (AFQAM) Training Conference, Lexington, KY, United States of America
13 Oct – 18 Oct 2019	Society of Forensic Toxicologists, San Antonio, TX, United States of America
24 Oct – 26 Oct 2019	28th Annual Congress of the European Association for Accident Research (EVU), Barcelona, Spain
29 Oct – 1 Nov 2019	International Association of Bloodstain Pattern Analysts (IABPA) Annual Conference, Chicago, IL, United States of America

The 10th AFSN Annual Meeting and Symposium 2018

Mr. Chen Song, Mr. Meng Qingzhen
Institute of Forensic Science, P. R. of China

The Asian Forensic Sciences Network (AFSN) held its 10th AFSN Annual Meeting & Symposium from 4 to 8 September 2018 at the Beijing International Convention Center, Beijing, China. The Symposium was officiated by Institute of Forensic Science, Ministry of Public Security, China, with the theme “New Technologies, New Methods, New Challenges”. Forensic science has complex interaction with science, technologies and society, and it has developed so much in the past decades and changed the way we pursue justice and against crime a lot, because of new technologies and methods continuously emerging. During the meeting, 564 colleagues from 24 countries gathered around to share knowledge and communicate, as well as to learn from each other. The meeting served as a good platform for technical communication, training, quality enhancement and strategy development for improving forensic science in Asia.

Lots of keynote speakers from different parts of the world came to the Meeting and shared achievements and experiences, such as Prof. Wang Guiqiang from IFS, Dr. Eliot Springer from NYPD crime lab, Dr. Mechthild Prinz from John Jay College of Criminal Justice, Dr. Gillian Treloar from The National Association of Testing Authorities, Australia gave speeches on the cold cases, forensic management, DVI and standardization. We had 10 keynote lectures and 96 oral presentations from the 6 workgroups, including, DNA, Illicit Drugs, Trace Evidence, Toxicology, Crime Scene Investigation and Digital Forensic. In addition, 31 posters were presented.



The key points that emerged from the meeting were that the new Workgroup of Digital Forensic successfully started. More than 60 delegates attended the Workgroup meeting and the chair, vice-chair and secretary of the WG were elected. Another important news was the setting up of Questioned Document Workgroup, initiated by IFS China, which was approved by the Board. The board members meeting also discussed some important issues of AFSN and agree new members to join AFSN. Now there are 53 member institutes from 15 countries. AFSN is speeding up its development.

This four-day symposium, ended with the lab tour, received encouraging response from not only the speakers but also the audience. Forensic scientists from Asia and beyond came together, shared, exchanged information and discussed collaboration, and had spent wonderful days in Beijing. The organizing committee wants to say thanks to all members that had given great support and friends that had attended.

The Birth of AFSN Questioned Document Workgroup

Dr. Han Xingzhou
Institute of Forensic Science, P. R. of China

Introduction

Questioned document examination (herein referred to as “QD”) is one of the oldest disciplines in forensic science, which uses scientific and technological methods to seek evidence that might be present in the document for investigation by the police officers and other agents, and to provide the evidence in the court of law. In every country, QD examiners can meet any types of documents, handwriting, typewriting, printing, seal, and forgery, fraud documents. A convenient definition of a document is something that contains information, usually in the form of paper. But nowadays, the document stored electronically is also needed to examine, who and how to examine is the topic which should be discussed and made further research.

The QD Workgroup

The Board of AFSN approved to create Questioned Document Workgroup (herein referred to as “QDWG”) in the 10th AFSN Annual Meeting & Symposium from 4 to 8 September 2018. It was initiated by IFS China, and now we are preparing its establishment, and has received positive responses from many countries, such as Singapore, Republic of Korea, India, Thailand, Malaysia and Sri Lanka. We have begun to draft a series of documents, such as Proposal of Establishment, Execution Plan, with assistance from the Secretariat of AFSN. The workgroup is aiming to promote the development of QD and facilitate the communication among the QD examiners and to promote the development and standardization of QD in Asia. We hope all the QD examiners of Asian can give more attention to QDWG, to work together to promote its growth. If you have any questions and good suggestions, please write to 11660920@qq.com.

The Birth of AFSN Digital Forensic Workgroup

Dr. Kang Yanrong, Mr. Zhang Yaoguo
Institute of Forensic Science, P. R. of China

The formation of Digital Forensic Workgroup (DFWG) was approved by the Board during 9th AFSN meeting, which was held in Singapore in September 2017. The Board decided that DFWG, being the 6th Workgroup in AFSN, would be initiated and constructed by IFS, China. From then on, DFWG has begun the preparation, communicating with experts within AFSN and discussing about needed documents.

After nearly one year of communication and preparation, DFWG workshop made a great success during the 10th AFSN Annual Meeting and Symposium in Beijing, which was organized by IFS from 4 to 8 September 2018. It attracted more than 60 experts, scholars, police, judges and researchers from 7 countries which included Sweden, Australia, South Korea, Thailand, Malaysia, Mongolia and China to attend the workshop. The academic reports and technical exchange activities of the DFWG covered the latest technologies in digital forensic field, such as drone forensics and automobile forensics. These new technologies reflected the future development direction. Certainly, it also included new methods applied in conventional digital forensic work, such as, digital data association mining under the big data framework, user behavior analysis based on audio data, application of video surveillance in scene exploration, new wearable digital device forensics and data analysis.

DFWG also had the first face-to-face business meeting on 6 September 2018, hosted by the chair Ms. Kang Yanrong, from IFS. More than 20 experts and scholars attended the meeting, representing 9 member institutes from 5 countries. Three vice-chairs and three secretaries were elected during the meeting. The participants spoke enthusiastically and put forward a lot of constructive ideas. In the end, we confirmed the members of DFWG, revised the charter of DFWG, and formulated next year's plan of DFWG. After the meeting, the DFWG will work as planned, and DFWG will hold a face-to-face meeting and technical discussion in Beijing in June 2019.

Development of ISO Standards in Forensic Science

Dr. Lui Chi Pang
Health Sciences Authority, Singapore

With the establishment of ISO/TC272 in 2012, an international effort has been under way to develop a set of ISO standards in forensic science to cater for laboratory and field based forensic science techniques and methodology, in the detection and collection of physical evidence, the subsequent analysis and interpretation of the evidence, and finally the reporting of the results.

Forensic science has played an important role in the administration of justice in our society. While the forensic community has been relying on ISO 17025 and ISO 17020 standards in establishing the laboratory quality assurance, they lack emphasis in some of the areas that are critical to forensic science, such as, chain of custody and peer review. The development of ISO forensic standards does not necessarily replace the ISO 17025 and ISO 17020, but can be used complementary to them or stand-alone.

The ISO/TC272 aims to achieve its objectives in enhancing the reliability of forensic evidence, establishing standard practices that can enable collaborative work from different jurisdictions in cross border investigations as well as supporting each other in the wake of disasters, exchanging forensic results and intelligence, ensuring forensic supplies are fit for purpose, and exchange of forensic professionals. In the development of these standards, the technical committee is mindful to include a wide range of disciplines that involve qualitative, quantitative and comparative testing, such as, drugs and toxicology, document examination, trace evidence, clandestine laboratory investigations, fire and explosion, ballistics, forensic biology, DNA, marks and impressions, fingerprints, crime scene investigation, entomology, anthropology, legal medicine, speech science and audio. However, digital evidence analysis and computer forensics are excluded.

Currently, ISO/TC272 comprises of a total of 42 participating and observing countries, with Australia holding the secretariat and chair. Out of the 23 participating countries which include countries from Europe, America, Oceania and Africa, only Japan and Singapore are the participating countries from Asia. Among the 19 observing countries, 6 of them are from Asia, including, China, Republic of Korea, Malaysia, Mongolia, Philippines and Thailand.

To date, 3 standards have been published, including, ISO 18385:2016 on “Minimizing the risk of human DNA contamination in products used to collect, store and analyze biological material for forensic purposes – Requirements”, ISO 21043-1:2018 on “Forensic sciences – Part 1: Terms and definitions”, and ISO 21043-2:2018 on “Forensic sciences – Part 2: Recognition, recording, collecting, transport and storage of items”. There are another 4 standards under development in the areas of Analysis, Interpretation, Reporting, and Specification for consumables used in forensic process – Requirements for product manufacturing and kit assembly.

Being part of the forensic community, AFSN member institutes in the participating and observing countries are encouraged to provide comments to the development of forensic science standards through their respective countries’ national standards bodies, so that AFSN members’ opinions can form part of this international effort in establishing an ISO standard that will be suitable for our region.

ISO/TC272 meeting in Stockholm, Sweden in May 2018.

Himachal Forensics in 21st Century

Dr. Arun Sharma
Directorate of Forensics Services, India

The Forensics Services Directorate has come a long way in Himachal since it came into existence on 13 December 1988, as a small unit based in the state police headquarters. It has been an eventful journey spanning over three decades synonymous with the evolution and growth of forensic services in the hill state. With a vast network of well-equipped forensic laboratories and specialties covering the whole range of contemporary forensic disciplines, the state today boasts of 'state-of-the-art" facilities, comparable with the best in the country.

Keeping in view the excellent work being done by the department and the growing importance of forensic services in investigation of crime, the government further plans to strengthen a full-fledged Directorate of Forensics Services to provide further impetus to this important and fast evolving field of crime investigation. It will be headed by Director General (Forensics) at the top with a Director for State FSL and Range Directors to oversee the functioning of various FSLs at the range level. The State FSL already has ten divisions and 14 specialties, including Biology & Serology, Chemistry & Toxicology, Document & Photo, Physics & Ballistics NDPS, DNA, Voice Analysis, Digital Forensics, Forensic Psychology and Finger Print Bureau. The implementation of the first 'Five Year Perspective Plan (2014 - 2019)' was an important initiative on the part of the department to develop modern infrastructure, new specialties, expertise, capacity building and requisite human resources. The plan was approved at the 8th Meeting of Himachal Pradesh Forensic Science Development Board.

Consequently, all types of forensic analysis facilities are now available under one roof and no case is required to be referred to any lab outside the state for analysis by the investigation agencies.

Likewise, at range level offices, divisions of Biology & Serology, Chemistry & Toxicology, Document & Photo, and Physics & Ballistics have been created. In addition, Cell Phone Forensic, Voice Analysis & DNA facilities have also been provided at range level as per demand of the investigation agencies. The State FSL and RFSL Northern Range are NABL ISO/IEC 17025 accredited labs. The State FSL & RFSLs have been examining & reporting 10,000 cases referred by the state & the Central government investigation agencies per annum.

It was the experience, intellect and vision of the founder Director of the State Forensic Science Laboratory that enabled him to lay a solid foundation of the institution. Pursuing the plans and the vision of the founder, the department has progressed to a level that today it was taking lead in the country in new emerging areas in forensics. One such initiative has been the creation of Disaster Victim Identification (DVI) cells, a first of its kind in the country, in accordance with INTERPOL guidelines.

In another first in the country, the department introduced expert testimony through video conferencing from the State FSL in 2015 and RFSL, Dharamshala, from 2018 to help save time of experts so that they can focus on the cases and have more time for lab work and other related activities.

In an endeavour to ensure quality of reports from private practitioners, the H.P. Forensic Document and Finger Print Examiners Regulatory Authority, 2017, was notified by the government with the Director of State Forensics Services, as the Chairperson. A Code of Ethics for forensics professionals was also approved.

The Government of India has also posed confidence in the forensic capabilities of the state and in a first in the country sanctioned funds under Prevention of Crime against Women & Children Scheme to establish two training centres (State FSL & RFSL Dharamshala) for the capacity building of various stake holders like police, prosecution and judiciary on cyber crime. The aim is to disseminate forensics knowledge to stake-holders of criminal justice delivery

system by holding regular training programmes, workshops and seminars. A booklet listing 'Do's & Don'ts' for the investigating officers was also published.

The Directorate has continuously been striving for acquiring latest know-how and its application in crime investigation to serve the society in the best possible way. The directorate is also working on a Memorandum of Understanding (MOU) with US Forensic Lab for professional joint exercises to attain international standards. A Memorandum of Understanding (MOU) has been signed with the Indian Institute of Technology, Mandi in 2017 for collaborative research and designing of tool which have applications in Forensic Science. The state government has supported its effort by promptly sanctioning Rs. 17 lakh ($ 23,789.53) from the State Innovation Fund.

The Directorate of Forensics Services (DFS) was recently conferred with membership of the Asian Forensic Science Network (AFSN) Board and became the first in the country amongst forensic science laboratories to have this honour. It will help in raising the standard of working and formulating strategies related to forensic science issues in Asia.

The directorate has been complimenting the state government's drive against narcotics and it has got the NDPS division of State FSL registered with the UNDOC to facilitate development of state-of-the-art lab facilities and collaborative studies in this important area.

The directorate has been making efforts to create awareness among people and regularly organizing exhibitions with a theme "forensics-in-action" during various fairs and festivals in the state, particularly the Red Cross Fair and International Fairs. Also, tableau is displayed during State level Republic Day celebrations since 2015.

Currently work is on preparing DNA Civil & Crime Data Base along with initiatives on humanitarian forensics and e-forensics projects so that forensic reports are delivered on line to the investigation agencies & the courts.

Introduction of the Institute of Forensic Science in Public Security Bureau of Suzhou

Dr. Li Min
Institute of Forensic Science, Public Security Bureau of Suzhou, P. R. of China

Established in 2001, the Institute of Forensic Science in Public Security Bureau of Suzhou (IFSSZ) specializes in the crime scene investigations, evidence examination, new technology research and development. The IFSSZ covers an area of 4500 m^2 and has around 37 staff members. It consists of 6 divisions and covers 16 forensic fields, including Forensic Pathology, Forensic injury, Forensic anthropology, Forensic Genetics, Toxicology and Drug, Fingerprint, Footprint, Tool Marks, Firearms, Bullets, Bombs, Image and video, Questioned Document and Digital Evidence. Our labs passed the China National Accreditation Service for Conformity Assessment (CNAS) in 2013, and obtained the China Metrology Accreditation (CMA) certificate in 2016. All the results are completed with strict QC procedures in order to ensure high quality.

Our main work is crime scene investigation and we investigate about 100 serious cases or matters every year, such as Huqiu “8.3” extraordinary murder case and Kunshan “8.2” Zhongrong explosion. The other part of our work is crime evidence testing. We test nearly 20,000 evidence every year, including the fingerprints, bloodstain, wiping samples and so on. We have built the DNA and the fingerprint database. The DNA database has more than 1,390,000 data and has more than 4,000 matched each year. The fingerprint database has more than 3,920,000 data and has more than 2,000 matched each year. Besides, we have accomplished more than 10 provincial and prefecture-level research programs. We have attained more than 20 Scientific Awards and 6 patents, such as Regional collaboration platform of the DNA database and 16CS Amplification Kit. In the future, we plan to put more people and financial resources in the scientific research, keep in touch with the AFSN members and hope to make cooperation with each other.

At IFFSZ, our mission is to make a positive contribution to society with science and technology to help create a safer world. We strive to advance the cause of justice with technologically advanced products and services, and we are also trying our best to continue to be a leader in conducting research and development in the areas of molecular biology and related advanced technologies.

Forensic Science Division of the Government Laboratory, Hong Kong Special Administrative Region (HKSAR), China

Mr. Bobbie Cheung Kwok Keung
Forensic Science Division of the Government Laboratory, HKSAR, P. R. of China

Government Laboratory of the HKSAR comprises two operational divisions; the Analytical and Advisory Division and the Forensic Science Division. Headed by the Government Chemist, the Laboratory is independent from all law-enforcement agencies; it provides a wide range of scientific services to various government departments to support the maintenance of law and order, public health and safety, environmental protection, government revenue and consumers' interests. It is a Designated Institute in the field of metrology for Hong Kong, China, in providing measurement traceability standards in Hong Kong and has demonstrated its high-level competency through inter-comparisons with national metrology institutes around the world. The Laboratory has been accredited as a proficiency testing scheme provider and reference materials producer since August 2006 and December 2010 respectively and has organized local, regional and international proficiency testing programs for testing laboratories.

Government Laboratory is one of the oldest organisations in the HKSAR Government with a history dating back 140 years to the appointment of the first Apothecary and Analyst in 1879. Since its establishment in 1913 as a separate analytical and medico-legal laboratory, it has provided Hong Kong with an ever-expanding range of scientific services. With the dramatic increase in its medico-legal role, a Forensic Division was created in 1969 incorporating all work relating to General Forensic, Narcotics and Toxicology. As a result of continual expansion in its work scope over the years, the Laboratory now occupies six satellite laboratories in addition to its Headquarters and has an establishment of over 480 staff.

The Forensic Science Division provides an impartial, comprehensive forensic scientific service to serve the Hong Kong Criminal Justice System. The Division, comprising 11 operational sections has an establishment of around 153 staff; over a third of whom are professional experts in various forensic disciplines. Its diverse work scope includes examination of DNA, contact evidence, physical evidence, controlled substances, questioned documents and toxicology. It also maintains a DNA database on behalf of Commissioner of Police for profiling and storage of DNA data of convicted offenders and suspects of serious criminal offences. About 350 round-the-clock scene attendances are made each year for client departments for major and minor crimes and specialised investigations such as causes of fires, re-constructing traffic accidents, blood pattern analysis and suspected illicit drug manufacturing and cultivation activities. Professional experts also provide 24-hour advisory assistance to law-enforcement agents on the scientific aspects of scene and exhibit examination to assist with criminal investigation and preliminary court proceedings as well as a 24-hour urgent laboratory examination service.

From 1996-2011, the Forensic Science Division was accredited by American Society of Crime Laboratory Directors / Laboratory Accreditation Board (ASCLD/LAB) under the Legacy Programme; since 2011, it has been accredited by Hong Kong Accreditation Service (HKAS) under ISO/IEC 17025. The transition to the ISO/IEC 17025 standard extended the accreditation scope for the Division to include non-crime testing areas such as hair drug testing in screening and monitoring the rehabilitation of drug-abusers.

The National Narcotics Laboratory of the National Dangerous Drugs Control Board, Sri Lanka

Mrs. Dona Pabasara Priyanganie Weerasinghe
National Dangerous Drugs Control Board, Sri Lanka

Introduction

The National Narcotics Laboratory (NNL) is a leading laboratory in Sri Lanka functioning under the National Dangerous Drugs Control Board (NDDCB), the national focal point for prevention and control of drug abuse in Sri Lanka. The NNL provides laboratory and scientific services for effective implementation scientific evidence based drug prevention and control system in Sri Lanka.

The NNL aims to better understand and monitoring the country's drug problem and improve scientific capacity and capabilities to ensure healthy lives and peaceful societies free from drug abuse.

The mission of the NNL of the NDDCB is to act as the principal scientific body with the responsibility of addressing the drug problem through an integrated, multidisciplinary, mutually reinforcing, balanced, coordinated and scientific evidence-based strategies implemented with shared responsibility which liaises with effective and increased national and international cooperation and coordination.

What we do?

- Scientific evidence generation for drug law enforcement operations and investigations
- Scientific evidence based strategic interventions
- Scientific intelligence services
- Promote scientific evidence based national policy and decision making
- Scientific consultancy services
- Scientific research
- Promote and advancement of science in drug control

Recent Developments

- Working Group for control of drug trafficking through internet and using postal services (WG-CIDTIP)
- Scientific laboratory network to tackle the emerging new synthetic drug threats
- Science, Technology and Innovation (STI) Platform for prevention and control of drug abuse to achieve the Sustainable Development Goals (SDGs)

Opportunities on National, Regional and International Coordination and Collaborations

- **Scientific staff capacity building programmes**
  The NNL has recognized the importance of a pool of young scientific community to implement innovative ideas and sustainable strategies to counter the dynamic nature of drug problem with mutual understanding and partnership with national, regional and international laboratories.

- **Collaborative Scientific Research on Challenging Problems in Drug Control**
  The NNL is engaged in scientific researches on narcotic drugs, psychotropic substances, New Psychoactive Substances (NPS) in order to address the emerging challenges collaboratively with national and international high profile researchers.

- **Networking of Scientific Laboratory Networks**
  The NNL gives highest priority to collaborate with regional and international drug testing laboratories for international harmonization of methods on laboratory testing and analysis, method developments and validation, sharing of scientific information on new drug trends and technical assistance to ensure the international standard laboratory practices and to be a competence partner within the Asia region.

- **Scientific communications for forensic society**
  The NNL regularly publishes the scientific research findings in local and international scientific journals, scientific recommendations in technical notes/guidelines and scientific advice to national policy makers and promote science to implementation the evidence based strategies in prevention and control of drug abuse.

Contact us

The National Narcotics Laboratory (NNL),
National Dangerous Drugs Control Board (NDDCB).
383, Kotte Road, Rajagiriya, 10107, Sri Lanka.
Tel: 0094-11-2868794, Fax: 0094-11-2868792
Email: nnl@nddcb.gov.lk
Website: www.nddcb.gov.lk

Introduction of Forensic Medicine Center of Ho Chi Minh City

Dr. Phan Van Hieu, M.D.
Forensic Medicine Center of Ho Chi Minh City, Vietnam

Forensic Medicine Center of Ho Chi Minh City was found in April 2007 with the primary mission to confirm the medical-legal aspects at the request of investigators, prosecutors or court following a judicial process. By Law of Judicial Expertise in 2013, FMC performs the living examination to estimate the permanent impairment, aging estimation, health assessment, sexual crimes, paternity, etc. as well as the medico-legal cases involving cause and matter of death.

At present, our center plays an important role in the leading group of medical forensic center not only in Ho Chi Minh City, but also in other 20 Southern provinces with whom we work in close collaboration. Ho Chi Minh City (HCMC) is a great urban center in the south of Vietnam, with over 13 million residents living on 2,061 square kilometres. As the highest population-concentrated city in Vietnam, beside many advantages in economy, culture, education and health services, we face off many challenges with traffic, environment and especially crime. Last year, in particular, our 50-staffed center accomplished our mission in the shortest time by running thousands of tests, ranging from imaging diagnosis to post-mortem toxicology, pathology and DNA to get the highest fidelity reports of more than 1,500 cases of living examination, more than 1,600 cases of autopsy and thousands of others.

Our center is also a forensic medicine training and educational center for medical universities such as: HCMC University of Medicine and Pharmacy and Pham Ngoc Thach Medical University, as well as National Institute of Forensic Medicine. Each year we receive hundreds of students per course and share with them our forensic science knowledge of autopsy, toxicology, DNA analysis, etc. We give them a friendly place where they do research and put in practice of what they have learned and what they should do in finding out the true cause of cases. Another primary activity we conduct is to send our people aboard to develop our knowledge, update the latest technical trends and innovative idea. For instance, we used to cooperate with Forensic Medicine Institute of Mainz, Germany in studying alcoholic metabolism gene, arrhythmogenic right ventricular cardiomyopathy condition.

We are proud of becoming the member of the Asian Forensic Sciences Network. We know that to do our mission in the best way not only in Vietnam but also in Asia and the world, we should work harder and promote scientific research by cooperating with other centers and institutes in Asia. Together, the medical doctors and scientists look for robust technique, methods to solve problems rapidly. For example, doctors discover a foreign body in the lung but traditional histology cannot confirm the kind of this substance. With the help of chemists using FTIR microscopy, we detect, name quickly that substance.

Evaluation of RSID™-Saliva and SERATEC® Amylase Test Kits for the Forensic Detection of Saliva

Ms. Wong Wai Ling, Mr. Lim Wei Siong Holden, Mr. Ping Yueh Shyang,*
Assoc. Prof. Syn Kiu Choong Christopher
Health Sciences Authority, Singapore
**Email: wong_wai_ling@hsa.gov.sg*

Abstract

The study evaluated two commercial kits, RSID™-Saliva and SERATEC® Amylase, for the detection of human salivary α-amylase in terms of sensitivity, specificity and interference. The results indicated that RSID™-Saliva has a higher sensitivity than SERATEC® Amylase, being able to detect as little as 0.125 µL of saliva. Both kits are specific as no false positives were detected from different biological fluids (blood, semen, and urine). Unlike SERATEC® Amylase, RSID™-Saliva was able to consistently detect saliva in 1:200 dilution (equivalent to 0.25 µL neat saliva) in the presence of 50 µL of blood, semen or urine. Overall, RSID™-Saliva demonstrated greater sensitivity, specificity and robustness compared to SERATEC® Amylase.

Introduction

Forensic detection of saliva can be beneficial in crime investigations to determine if saliva was deposited on evidence such as bottles and envelopes. Traditional forensic saliva detection methods generally examine for the enzymatic activity of α-amylase or the fluorescence of saliva as a biological fluid. Such assays include the Phadebas® amylase test, which gives false positive results in the presence of hand creams, lotions or washing powders [1], and starch-iodine colorimetric tests which also yield false positive results if blood and semen are present. Under an ultraviolet light source, saliva would glow as with other biological fluids [2]. While these tests are simple to perform, they are non-specific, laborious and do not integrate well with DNA-based protocols. Newer commercial assays, such as the RSID™-Saliva and SERATEC® Amylase kits, are lateral flow immunochromatographic strip tests that utilize two anti α-amylase monoclonal antibodies to detect the presence of human salivary α-amylase rather than its activity. These kits are quick and easy to use, offering a higher level of specificity as the amylase-specific antibodies recognise the salivary α-form, not the pancreatic α-form or β-form found mainly in plants and fungi [3]. As such, an evaluation of both kits was conducted to assess their suitability for use in casework.

Materials and Methods

Sample preparation

Two saliva detection kits, SERATEC® Amylase (Goettingen, Germany) and the RSID™-Saliva (IL,USA) were used. Each biological fluids (blood, saliva, semen, breast milk and urine) was obtained from 3 volunteers. All stained samples were dried overnight before processing.

Sensitivity studies

Samples were prepared by staining sterile cotton buds with 50 µL of saliva diluted with ultrapure water (1:50, 1:100, 1:200, 1:400).

Specificity studies

Biological fluids (blood, semen, breast milk and urine) were used. Samples were prepared by staining sterile cotton buds with 50 µL of neat biological fluid (blood, semen, breast milk and urine).

Interference studies

50 µL of various neat biological fluids (blood, semen, and urine) were added onto cotton buds stained with 50 µL of diluted saliva (1:50 and 1:200).

RSID™-Saliva testing

Stained swabs were excised and incubated in 200 µL of RSID™-Universal Buffer with agitation at 1,000rpm at a constant 25°C for 30 minutes. After incubation, 20 µL of sample extract was diluted in 80 µL of RSID™-Universal Buffer. The final volume of 100 µL was loaded onto the cassette and results were read at 10 minutes after loading.

A positive detection was observed when red lines were detected at both the test and control regions of the cassette. A negative detection was observed when a red line was detected at the control region but no red line observed at the test region. The result was deemed as invalid when no red line was detected at the control region of the cassette.

SERATEC® Amylase testing

Stained swabs were excised and incubated in 200 µL of AMY Buffer Solution with agitation at 1000rpm at constant 25°C for 30 minutes. After incubation, 24 ul of sample extract was diluted in 96 µL of AMY Buffer Solution. The final volume of 120 µL was loaded on to the cassette and results were read 10 minutes after loading. The interpretation of results is the same as RSID™-Saliva testing.

DNA analysis

DNA extraction was performed on 5 saliva sample extracts in RSID™-Universal Buffer using the DNA IQ™ Casework Pro Kit on a Maxwell® 16 instrument (Promega Corp., Madison, WI). Extracted DNA was quantified on 7500 Real-Time PCR Instrument (Applied Biosystems, Foster City, CA) using Quantifiler® Duo DNA kit. STR-PCR amplification was performed with Globalfiler™ PCR kit. Amplicons were analyzed on the 3500xL Genetic Analyzer with data analyzed using Genemapper®IDX ver 1.2 software. Extraction, quantification, amplification and capillary electrophoresis were all conducted in accordance to manufacturer's protocols.

Results

Sensitivity studies

RSID™-Saliva was able to detect α-amylase down to 1:400 dilution, which is equivalent to 0.125 µL of neat saliva. In comparison, the SERATEC® Amylase was only able to detect α-amylase down to 1:200 dilution (Table 1).

Specificity studies

No α-amylase was detected in blood, semen and urine using RSID™-Saliva and SERATEC® Amylase (Table 2). However, both kits detected α-amylase in breast milk. This is likely to be sample inherent as α-amylase has been reported to be present in breast milk [4].

Interference studies

RSID™-Saliva kit was able to consistently detect α-amylase at both 1:50 and 1:200 dilutions in the presence of 50 µL of neat blood, semen and urine (Table 3). In comparison, the SERATEC® Amylase kit could not detect α-amylase at 1:200 dilution in the presence of semen. Presence of urine also interfered with the detection of α-amylase at both 1:50 and 1:200 dilutions.

Dilutions	Results	
	RSID™-Saliva	***SERATEC® Amylase***
Neat	Positive	Positive
1:50	Positive	Positive
1:100	Positive	Positive
1:200	Positive	Positive
1:400	Positive	Negative

Table 1. Sensitivity of both kits (n=3)

Biological fluid	Results	
	RSID™-Saliva	***SERATEC® Amylase***
Blood	Negative	Negative
Semen	Negative	Negative
Urine	Negative	Negative
Breast milk	Positive	Positive

Table 2. Specificity of both kits (n=3)

Biological fluid	Saliva dilution	Results	
		RSID™-Saliva	***SERATEC® Amylase***
Blood	1:50	Positive	Positive
	1:200	Positive	Positive
Semen	1:50	Positive	Positive
	1:200	Positive	Negative
Urine	1:50	Positive	Negative
	1:200	Positive	Negative

Table 3. Interferences by other biological fluids for both kits (n=3)

DNA analysis

Concordant DNA profiles were obtained from the saliva extracts of 5 donors, indicating that the RSID™-Universal buffer is compatible with DNA extraction. A representative profile is shown in Figure 1.

Conclusion

The present study indicated that both RSID™-Saliva and SERATEC® Amylase kits can be used to examine for the presence of α-amylase. However, the RSID™-Saliva was shown to be more sensitive and less affected by presence of biological fluids such as semen and urine. Additionally, the products of the RSID™-Saliva testing were shown to be compatible with downstream DNA extraction processes. The Laboratory has since adopted RSID™-Saliva as the test method for the forensic detection of saliva in casework.

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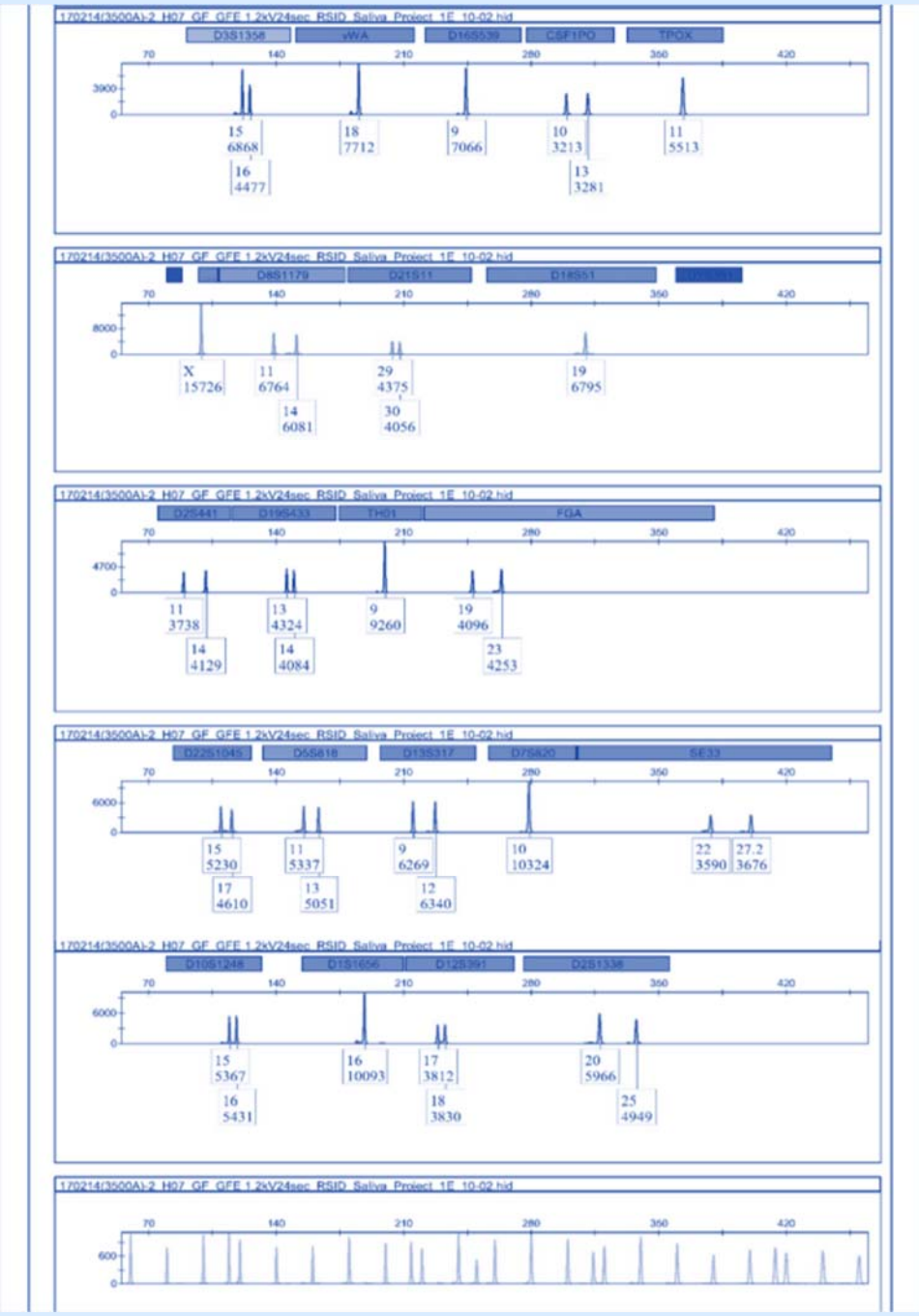

Figure 1. DNA profile obtained from the saliva extracts (n=5)

A Comparison of Extraction Methods for Urine Sample

Mrs. Nor Ummiza Kamaruzaman, Mr. Mohd Firdaus Che Amran, Mr. Mohd Azhar Mat Zin, Mrs. Aidora Saedon, Dr. Cornelia Charito Siricord*
Department of Chemistry, Malaysia
**Email: norummiza@kimia.gov.my*

Introduction

Urine is a body fluid or secretion that has nucleated cells containing DNA which can be isolated and analyzed [1]. DNA in urine comes from epithelial cells that line the bladder and ureters. Urine, recovered from crime scenes such as abuse, sexual assault and kidnapping, is among the most important evidence to forensic investigators [2]. Although urine is not considered as an ideal source of DNA due to the low concentration of nucleated cells, it still contains valuable DNA evidence which can identify or exonerate a person [3].

The objective of this study is to evaluate four different methods of DNA extraction from human urine. The two main methods regularly used are chelex and organic extraction methods. The Applied Biosystems Automate Express™ Forensic DNA Extraction System using the PrepFiler Express™ Forensic DNA Extraction Kits is selected as the latest automation technology. Urine was also spotted onto FTA® Cards and extracted according to the Life Technologies protocol. Each of the extracted samples was quantified using the Quantifiler™ Human DNA Quantification Kit with Applied Biosystems RT-PCR 7500 System to assess the amount of DNA. The DNA yield was analysed by Applied Biosystems 3130xL Genetic Analyzer and GeneMapper™ ID Software was used to generate the DNA profile.

Materials and Methods

Urine samples of four volunteers [healthy man, healthy woman, healthy child and senior citizen with medication] have been collected. Samples were divided into three different groups. First group was immediately analysed. Second group was stored for one month and third group was stored for two months in a cold room at 4°C. 50 mL of urine was used in all the methods except for FTA. For each method, the analysis was done in triplicates.

<table>
<tr><td>Sample Prior extraction</td><td colspan="3">Urine pellet</td><td>Liquid Urine</td></tr>
<tr><td rowspan="2">Extraction Method</td><td>Chelex Extraction</td><td>Organic Extraction</td><td>Automate Express System</td><td rowspan="2">FTA Direct Method</td></tr>
<tr><td colspan="3">Quantifiler™ Human DNA Quantification Kit with Applied Biosystems RT-PCR 7500 System.</td></tr>
<tr><td>Amplification Kit</td><td colspan="3">AmpFISTR® Identifiler® Plus</td><td>Identifiler® Direct PCR</td></tr>
</table>

Figure 1: Methods involved in this study

Urine cell pellet was prepared from urine samples collected in specimen bottles. The urine samples were first inverted to create a homogeneous suspension of cells and subsequently transferred into a tube and centrifuged for 3 min at 1,000 rpm to pellet all corpuscular bodies, cells and parts of cells. The supernatant was removed and the remaining dry pellet was chilled. This process was repeated until the entire urine volume was used up.

Chelex Extraction

Chelex extraction was performed on urine cell pellet. 150 µL of 20% chelex and 4 µL of (10 mg/mL) Proteinase K was added to the suspended urine pellet and incubated at 56°C for at least 2 hours. The tube was then vortexed and incubated at 100°C in a thermoblock for 8 minutes. The tube was subsequently vortexed and centrifuged for 3 minutes at 13,000 rpm. The extracted samples were stored at 0-4°C.

Organic Extraction

Organic extraction was performed on urine cell pellet. Urine pellet was digested with 500 µL digestion mixture (10 mM Tris-HCl - pH 7.5, 10 mM EDTA, 50 mM NaCl and 20% (w/v) SDS) and 25 µL Proteinase K at 56ºC for at least 5 hours. 0.4 mL buffered phenol-chloroform-isoamyl alcohol solution was added to the sample and vortexed for 2 minutes. The sample was then centrifuged for 5 minutes at 13,000 rpm at room temperature and the upper aqueous layer was

transferred to a fresh sterile 1.5 mL micro centrifuge tube. The steps were repeated from the addition of buffered phenol-chloroform-isoamyl alcohol solution until the aqueous layer was clear. DNA was then precipitated with 1.0 mL of absolute ethanol and the tube was inverted several times to mix before storing at -20ºC for at least one hour. The DNA precipitate was then pelleted down after centrifugation for 20 minutes at 13,000 rpm. The supernatant was decant and any remaining liquid was pipetted out. The DNA was vacuum-dried by centrifuging in a speed vacuum concentrator at low to moderate heat. Lastly, the DNA was reconstituted in 40 µL of TE buffer. The extracted samples were then stored at 0-4°C.

Automate Express System

Two types of samples were used for the Automate Express system: urine pellet and liquid urine. 500 µL of the lysis solution was added into the sample tube. The sample was incubated in a thermomixer at 70°C and 750 rpm for 40 minutes. The lysate was purified using PrepFiler Express™ Forensic DNA Extraction Kits according to manufacturing protocol. The extracted samples were then stored at 0-4°C.

FTA paper

50 µL of urine was spotted onto FTA paper and dried, punched out using the Harris Micropunch and ejected into a 1.5 mL microcentrifuge tube. 200 µL of 10 mM NaOH was added to the sample and gently shaken on a vortex for 5 minutes. The alkaline wash solution was discarded. 200 µL TE buffer was added to the washed disc and shaken gently for 5 minutes. The wash buffer was then discarded. 200 µL sterile distilled water was added and the sample was rinsed for 5 minutes on the vortex mixer. Discard the last rinse.

Results and Discussion

This study demonstrated a comparison of methods for obtaining DNA from urine samples which can be used for DNA profiling. Figure 2 shows the mean yield of extracted DNA concentration from the triplicates, determined by the RT-PCR 7500 System. No profile was detected using FTA method from urine which is immediately analysed. It shows that the FTA method was not suitable for this urine study. Hence, FTA method was not performed for the other groups of samples. Organic, Chelex and Automate Express extraction methods all gave full DNA profiles, with the exception of direct urine samples stored for two months using the Automate Express method. The highest mean DNA yield obtained was 5.68 ng/uL (organic extraction-urine pellet).

Extraction method		Chelex extraction	Organic extraction	Automate Express System	
Urine prior extraction		Urine pellet			Urine
Healthy Man	Immediately	0.40	0.21	1.82	0.011
	1 month storage	0	0	0	0
	2 month storage	0.19	0.23	0.07	0
Healthy Woman	Immediately	0.10	**5.68**	0.79	0.001
	1 month storage	0.22	1.61	0.43	**0.037**
	2 month storage	0.20	0.63	0.01	0
Healthy child (male)	Immediately	0.15	1.10	1.06	0.016
	1 month storage	0	0.02	0	0.010
	2 month storage	0	0.15	0	0
Senior citizen with medication (female)	Immediately	**0.96**	3.26	**1.89**	0.029
	1 month storage	0.07	0.69	0.01	0
	2 month storage	0.94	2.10	0.14	0

Figure 2: Mean concentration (ng/uL) of DNA yield.

Figure 3 shows DNA profiles of senior citizen with medication obtained from chelex extraction, organic extraction and Automate Express System respectively. These three representative profiles were the DNA extraction from urine pellet stored for one month at 4°C using the respective methods.

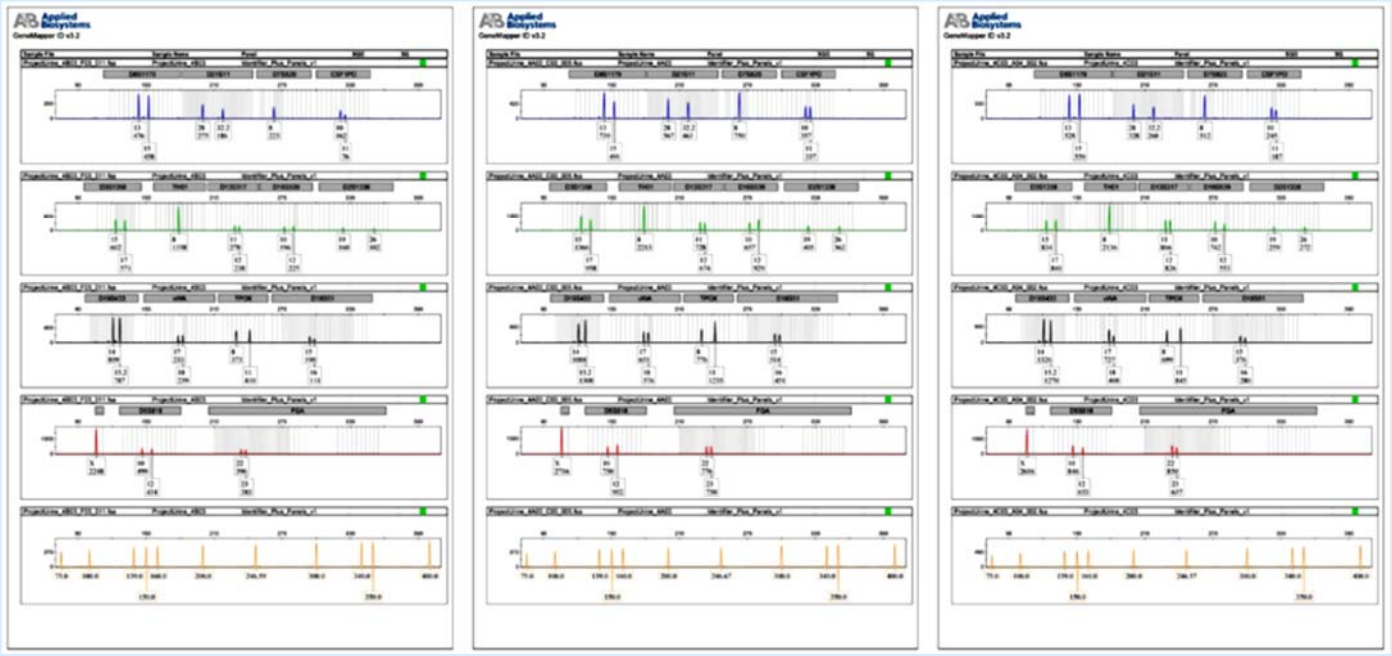

Figure 3: Representative DNA Profiles from this study.

The results show that liquid urine, which is not pelleted, and stored for a longer duration, exhibits lower concentration of DNA, leading to no DNA yield. It suggests that pelleted urine is a more reliable way to gain higher DNA yield. No DNA profile was detected when using automated system to extract DNA from direct urine sample after two month storage. Due to naturally produced bacteria in the urine, it is likely that longer storage will degrade the DNA [1].

Forensic analysis involving a small volume of encountered urine requires a rapid, easy and cost-effective analysis. From this study, though organic extraction yields the most DNA from human urine, this method is time-consuming and involve highly toxic reagents. Hence, the preferred method is the automated system which also provides the benefits of minimizing contamination and effectively isolates high quality genomic DNA. DNA obtained from the automated system gave complete result of short tandem repeat (STR) profiles, which was conclusive and devoid of PCR artefacts, and this is similar to observations in a previous study [4].

Conclusion

Urine sample was successfully extracted using organic, chelex and Automate Express System. Stained urine on FTA card was unable to give any DNA yield or DNA profile, suggesting this is not an appropriate method for obtaining DNA profile from urine samples. Pelleted urine gave more DNA yield compared to non-pelleted liquid urine. Urine samples which were immediately analysed generally yielded more DNA compared to those which were stored for long term. Organic extraction was the best method for yielding high DNA from urine samples, followed by Automate Express System and chelex extraction.

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Research Progress on Age Estimation of Fingermarks by the Components Analysis

Ms. Sun Jing [1,2], Ms. An Ran[2], Mr. Zhong Tao[2]*
[1] China University of Political Science and Law, P. R. of China
[2] Beijing Public Security Bureau, P. R. of China
**Email: sunjing102003@126.com*

Abstract

Aging of fingermarks could have a significant impact in forensic science, as it has the potential to facilitate the judicial process by assessing the relevance of a fingermark found at a crime scene. During recent years, scientists have done a lot to research this issue. This article attempts to introduce researchers progress on aging fingermarks by using advanced instruments.

Determination of the time since deposition of fingermarks may prove necessary to link their pertinence with certain crimes and criminals. Up to now, there are no methods that can reliably predict the age of a latent fingermark because various factors, such as different donors, diverse carriers and changing environment, can affect the age estimation of fingermarks. In the past, age has been estimated from the quality of the print and the ease of development with, for instance, dactyloscopic powder, but fingermarks appearing “fresh” were in fact old.

The main progress made so far is classifying fingermarks as younger, or older than 5h using white-light imaging. With the development of more sensitive instruments and devices, researchers have tried many approaches to solve the problems. Mass spectrometry (MS) of latent fingermarks has been an area of increasing interest due to the enormous amount of chemical information that can be extracted regarding the donor.

Fingermarks contain both endogenous compounds such as fatty acids, sterols, squalene, and wax esters that can be used to identify the biology of the donor such as age and gender as well as exogenous compounds such as cosmetics, drugs, and explosives that can be used to connect the donor to a crime scene. Attributing spatial information to such compounds provides new perspectives to latent fingermark analysis that is relevant to forensics, such as the activities of the donor prior to or during deposition of the fingermarks, which may potentially aid in the reconstruction of a crime scene or may assist in its tactical investigation.

Fingermarks’ oxidation processes have been proposed to be used to estimate their age. According to the references, tryptophan-containing proteins (Tryp) have been suggested to be the main contributor to fresh fingermarks autofluorescence. When exposed to air, unsaturated lipids oxidize and form reactive oxidation products (LipOx), which react with proteins to form fluorescent oxidations products (FOX). Fluorescence originating from non-protein fluorophores may lead to a Tryp overestimation. Annemieke [1] tried to construct a reference spectrum using six fingermarks washed with a chloroform/methanol mixture known to elute fluorescent oxidation products and other unknown fluorescent components while retaining proteins. For age estimation, the Tryp/FOX ratio was measured for the first time after fingermark deposition and subsequently for several days. The ratio has been used successfully to estimate the age of 55% of the fingermarks up to three weeks old from male donors, with an uncertainty of 1.9 days.

The composition of fingermarks’ residues is too variable to allow any prediction about the age of fingermarks within reasonable boundaries. Champod et al. have pointed out that by the ratio of the target component’s content degrading over time divided by the sum of more stable compounds (which are less variable from print to print) in fingermark residues, the time when a fingermark was deposited can be inferred [2]. They used GC/MS to study the composition of fingermark residues. Squalene and cholesterol were identified in fingermarks of all donors, which additionally contained several squalene derivatives, fatty acids, and wax esters. A solution was found to address this variability issue by using relative peak area definitions between compounds inherent to fingermarks. For example, using the relative peak area of squalene to cholesterol, the relative standard deviation (RSD) of the relative amounts could be reduced substantially. This variation was, however, still significant for fingermarks from the same donor deposited on different days.

The model time of flight secondary ion imaging mass spectrometry (TOF-SIMS) imaging system has been demonstrated for the age dating of fingermarks. Shin Muramoto et. al. proposed that mass spectrometric imaging of latent fingermarks is an area of increasing interest due to the enormous amount of chemical information that can be extracted regarding the donor [3]. The rapid acquisition of large, spatially resolved images with monolayer sensitivities were useful for the visualization of the diffusion of sebaceous molecules originating from the fingermark. According to the paper, a fingermark less than 4 days old could reliably be deduced through the extent of diffusion of palmitic acid.

Conclusion

Previous research has shown that the composition of fingermarks' residues is too variable to allow any prediction about the age of fingermarks within reasonable boundaries. This paper reviewed studies using advanced instruments to develop reliable methods. Further studies, including alternative analytical methods, such as desorption electrospray ionization mass spectrometry (DESI-MS), should aim at identifying other potential intrinsic target compounds to get more reliable methods of aging the fingermarks.

Acknowledgments

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Simultaneous quantification of Δ9-THCA-A, Δ9-THC, CBN and CBD in seized cannabis using UPLC/PDA-QDa

Weilai Sun[1], Xiaoyu Zheng[2], Yanbiao Zhao[2], Lisheng Gao[2], Hui Zheng[2]
[1] *People's Public Security University of China, P. R. of China*
[2] *Institute of Forensic Science, Ministry of Public Security, P. R. of China*

Abstract

An analytical method using ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC/PDA-QDa) for the qualitative and quantitative analysis of Δ9-tetrahydrocannabinolic acid-A (THCA-A), Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) in confiscated cannabis plants has been developed. The seized cannabis plants were extracted in methanol by sonication. After centrifugation, the supernatants were separated on a Waters UPLC BEH C_{18} column (100 mm×2.1 mm, 1.7 μm) with isocratic elution at a flow rate of 0.2 mL/min, coupling with PDA at 220 nm and confirmed by mass spectrometer QDa. The correlation coefficient of standard curve for each cannabinoid in linearity range was not less than 0.999, as well as the recoveries of each analyte in cannabis were 82%-102% with the relative standard deviations (RSDs) of 0.61%-4.12%. The analysis of the original composition of plant material is necessary for the detection and the quality control of cannabis plants. The results showed that the total THC content obtained by UPLC method (Δ9-THC+Δ9-THCA-A×314/358) was slightly higher than that of GC method. The method with simple pretreatment measures was specific, easy, accurate, reproducible and suitable for confirmation of the cannabinoids in seized cannabis.

Introduction

Cannabis (*Cannabis sativa* L.) is a plant controversially discussed worldwide and its cultivation is prohibited in most countries. Some countries have reported illegal growth and traffic of the herbal products [1-4], since cannabis is the most frequently used illicit drug in the world. More than 500 different chemical substances have been isolated from the plant, of which cannabinoids are the most studied and well known cannabis compounds [5-7]. The main biologically active cannabinoid is Δ9-tetrahydrocannabinol (THC), about 90% of the total THC is available as Δ9-tetrahydrocannabinoic acid A (THCA-A) in fresh plant material. THCA-A is the non-psychoactive precursor of THC [8]. Other constituents are present in significant amounts, including cannabinol (CBN) and cannabidiol (CBD). As THC is thermolabile and photolabile, the storage of cannabis leads to a cumulative decrease in THC content through oxidation of THC to CBN [5]. The contents of every cannabinoid can vary depending on the storage conditions such as light and temperature factors.

The cannabis plants are differentiated and classified into drug-cannabis (resinous cannabis) and fiber-cannabis (textile cannabis), according to the THC concentration in the dry flowering tops of cannabis plants. If THC exceeds 0.3%, the plant is classified as "drug phenotype" [9]. Whereas the European Union allows cultivation of fiber varieties with THC content of less than 0.2% [10]. According to the UNODC, the maximum permitted content in fiber cannabis varieties is 0.3% THC [11].

To evaluate the chemical potency of cannabis requires the measurement of the total THC content comprising the sum of the free THC and its precursor THCA-A. This is because THCA-A converts into the psychoactive THC when heated or smoked [12]. The thermal conversion of THCA-A occurring in gas chromatography (GC) systems was demonstrated to be incomplete and irreproducible [12,13]. GC could not detect THCA-A because of decarboxylation at the temperature of injection port. With liquid chromatography (LC), samples are not heated. THCA-A and neutral THC are therefore detected, and their concentrations are summed to obtain the total THC content [14]. LC is a suitable alternative that allows the analysis of both free cannabinoids and their acids in the same run without the need of derivatization [15].

An ultra-high performance liquid chromatography with photo-diode array detection tandem mass spectrometry with quadrupole Dalton detection (UPLC/PDA-QDa) for the quantitative detection of Δ9-tetrahydrocannabinolic acid-A (THCA-A), Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) in confiscated cannabis plants has been developed, fully validated and applied to analyze seized cannabis products. Analytical procedures require the developments of new technique in order to improve the detection and separation of all cannabinoids present in plant tissues. UPLC method has been developed to determine the cannabinoid pattern in cannabis. UPLC detects cannabinoids in both the acidic and neutral forms and provides an effective tool for more accurate assessment.

Material and Methods

Materials used: methanol (Fisher Scientific, American), acetonitrile (Sigma, American), ultra-pure water (Millipore, American), reference standards: THCA -A, THC, CBD and CBN as solutions 1 mg/mL used for preparation of stock solution (supplied by Cerilliant, American).

Sample preparation

The illicit herbal cannabis plants seized by police authorities in different provinces of China were sent to Institute of Forensic Science and Ministry of Public Security for forensic chemical analysis. The upper part (about 10 cm) of the main stem of cannabis plant was chosen and was first dried at room temperature (20± 2°C) in darkness and then was ground in a high-speed universal grinder (Tai Site Instrument Co., Ltd., TianJi, China) followed by further homogenization. Each sample consisted of 10 mg of the powder and was suspended in 5 mL of methanol. The sample was vortexed for 30s and extracted by ultrasonication for 30 min. Subsequently the sample was centrifuged at 6000 r/min for 5 min by a desktop high-speed refrigerated centrifuge (Sigma, Germany). The supernatant was then passed through a 0.22 μm needle type filter (Shimadzu-GL, Shanghai) yielding a sample ready for injection.

Analytical method

For analysis, an ACQUITY UPLC H-Class with photo-diode-array detector (PDA, Waters) tandem mass spectrometry with quadrupole Dalton (QDa, Waters) dectection (UPLC/PDA-QDa) was used. Chromatographic separation was achieved with the following column: Waters ACQUITY^{TM} UPLC BEH C_{18} (50 mm x 2.1 mm, 1.7 μm). Mobile phases consisted of: A: ultra-pure water, B: methanol (containing 0.1% formic acid), isocratic separation with 13%A and 87%B, flow rate: 0.2 mL/min. Injection volume: 2 μL. Detection was at wavelength of 220 nm. For seal wash of the injection port, 10% methanol in ultra-pure water was used.

The mass spectrometer was operated for the simultaneous separation and identification of cannabis constituents. MS acquisition was carried out in the positive and negative ESI mode. The conditions were set as follows: The scanning mass range was from 100 to 500 Da (m/z). The sample from the ultra-high-performance liquid phase went directly to the mass spectrometry QDa, sampling rate of 10 dots/s, a capillary voltage of 0.8 kV, a taper hole voltage of 30 V and a probe temperature of 600°C.

The quantification of the extracts by means of UPLC/PDA-QDa was performed with external calibration, a set of standards containing target compounds at concentrations range in MeOH were analyzed in the same conditions of the samples. The calculation of total THC content is according to the conversion factor of THCA-A to THC (MW_{THC}/MW_{THCA-A}=314.47 g.mol^{-1}/358.48 g.mol^{-1}=0.88) [8]. Data processing was performed using Microsoft Excel 2007.

Method validation

For THC, CBD and CBN a six point calibration curve with the following concentrations were prepared: 0.5, 1, 2, 5, 10 and 20 μg/mL in methanol. Because of the higher concentration of THCA-A in samples a separate six point calibration curve with the following concentrations was used: 2, 5, 10, 20 and 40 μg/mL in methanol. Calibration samples were run on a weekly basis.

Selectivity and specificity were assessed by analysing blank samples (methanol) and the lowest calibrators. Limits of detection (LOD) and limits of quantification (LOQ) were calculated from averaged data of six different calibrations. Precision and accuracy were calculated by analysing cannabis samples at least six times on different days.

Results

Method validation

The chromatographic system was based on methanol (0.1% formic acid)-water, allowing to reduce the damage to the environment, and 1.7 μm C_{18} reversed phase material, allowing to reduce the flow rate to 0.2 mL/min. A sharp symmetrical peak-shape and sufficient separation of the cannabinoids from the complex plant matrix can be achieved. Peak identification was achieved by standards and their characteristic PDA spectra. No interference was visually observed at retention time of analytes in blank. The main cannabinoids were completely separated in less than 7min.

The selectivity of detection of each compound was ensured by the determination of the retention times and the recording of the complete PDA spectra of the cannabinoids. The quantitation analyses were carried out using PDA at 220 nm for the analytes (Figure 1). The combined QDa analysis was performed to aid in peak identification. The purity for each compound was checked by QDa in either positive or negative modes. No other components or contaminants in cannabis extracts were detected at the wavelength within the retention time of each cannabinoid.

QDa instrument offered high selectivity under full-san conditions compared to other constituents. The ESI (+) showed the characteristic protonated molecular ion expected for CBD, CBN and THC (Figure 2 a-b and d-e). Thus, extracted ion chromatogram (EIC) of ions 315 (CBD and THC) and 311 (CBN) were selected for monitoring those cannabinoids.

The ESI (-) provided qualitative analysis of acidic cannabinoid THCA-A (Figure 2 c and f). Therefore, the EIC of ion 357 were used to confirmation of THCA-A. Mass spectrometric date for analytes are in good agreement with UPLC/PDA analyses.

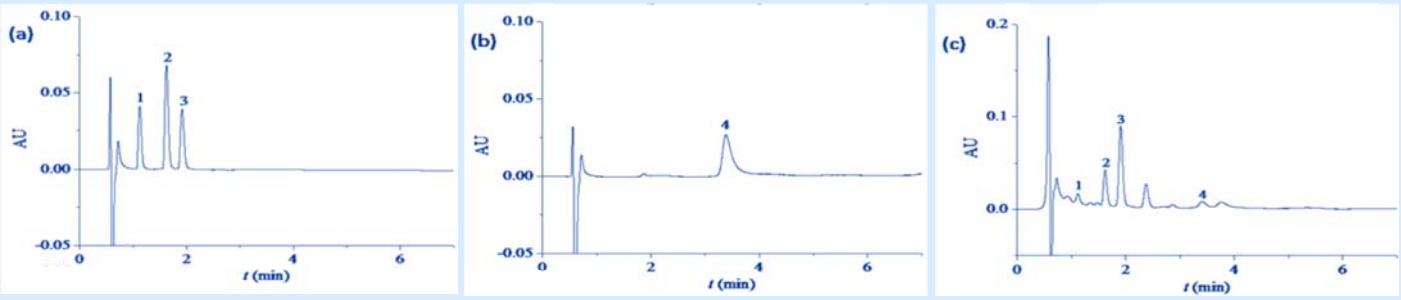

Figure 1: Typical chromatograms for the reference standards CBD, CBN and THC (a) and THCA-A (b) which are measured in two separate runs, since concentrations are different in the calibration sets (run time 7 min). (c) Typical chromatogram of an authentic sample.

Peaks: 1, CBD 1.129 min; 2, CBN 1.630 min; 3, THC 1.924 min; 4, THCA-A 3.405 min.

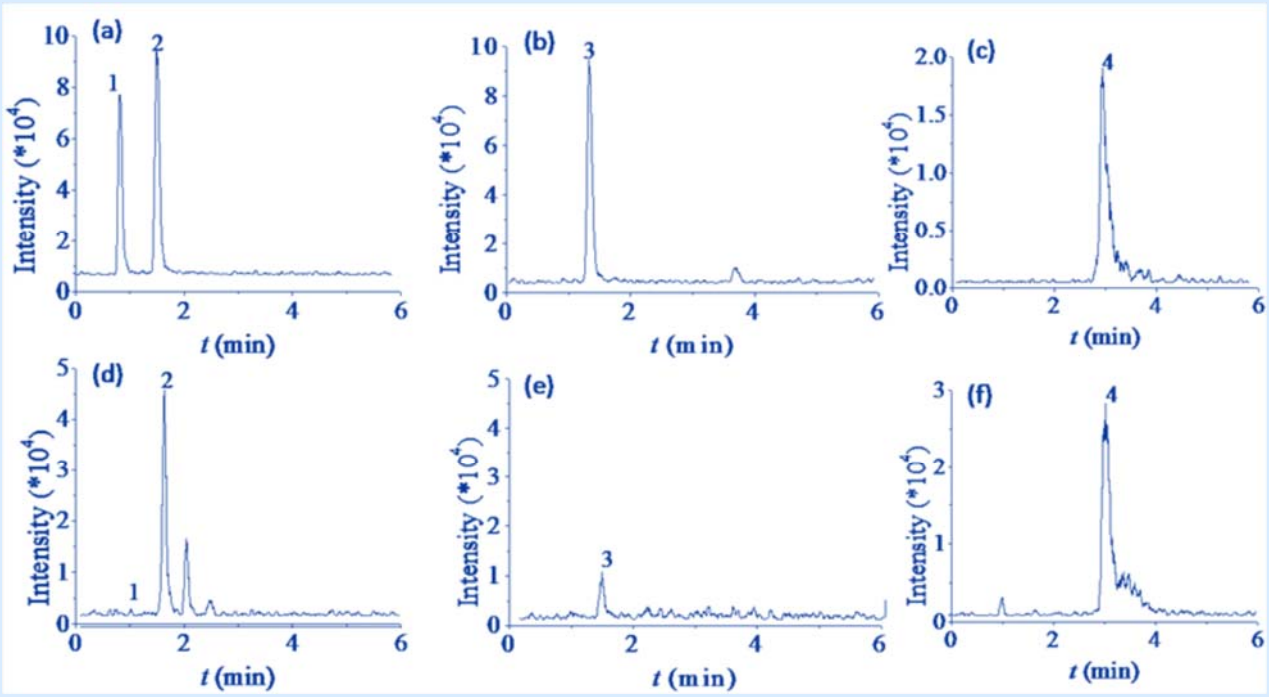

Figure 2: Extracted ion chromatograms of standard substances (a-c) and cannabis samples (d-f) by QDa confirmation analysis.
Peaks: 1, CBD (m/z 315, ESI⁺); 2, CBN (m/z 311, ESI⁺) ; 3, THC (m/z 315, ESI⁺); 4, THCA-A (m/z 357, ESI⁻)

The selectivity can be enhanced by improving the chromatographic separation between the analyte and the interference using PDA. Therefore UPLC coupled with QDa detector will have a good prospect in the cannabis field.

The limit of detection (LOD), experimentally measured with standard mixtures, was each cannabinoid on the chromatographic system with a signal to noise ratio of 3:1 or higher (s/n≥3). The limit of quantitation (LOQ) was determined from the signal-to-noise ratio of 10:1 (s/n≥10). Calibration curves were built in the range of 0.5-20 µg/mL for THC, CBN, CBD and 2-40 µg/mL for THCA-A.

The linear relationship was evaluated between the peak area and the concentration of each cannabinoid. The correlation coefficient R and linear equation of each cannabinoid is shown in the Table 1. The calibration parameters were stable with regression coefficients always >0.999 for each cannabinoid. The curves were linear in the concentration range for each analyte.

Component	Linear range (μg/mL)	Regression equation	LOD (μg/mL)	LOQ (μg/mL)
CBD	0.5-20	Y=4.00×10^4 X-1.06×10^3 （R=0.9999）	0.1	0.4
CBN	0.5-20	Y=6.64×10^4 X+3.44×10^3 （R=0.9998）	0.05	0.2
THC	0.5-20	Y=3.76×10^4 X-1.15×10^3 （R=0.9999）	0.1	0.4
THCA-A	2-40	Y=3.84×10^4 X-2.03×10^3 （R=0.9998）	1	2

Table 1: Linearity, Limits of detection (LODs) and limits of quantification (LOQs) for each cannabinoid.

The intraday precision of the method was assessed by applying the procedure repeatedly to multiple samplings (n=6) of one sample within one day. The interday precision was checked by analyzing repeatedly the same sample at 6 different days within one week. The relative standard deviation (RSD) for the intraday and interday variation was calculated respectively (Table 2).

The accuracy of the method was determined by spiking one cannabis extract with a known amount of cannabinoid standards. The accuracy was acceptable for all cannabinoids, since the relative bias (%) were always smaller than 4.12%. Results are presented in Table 2.

Component	Precision (%)		Accuracy (%)	RSD (%)
	Intra-day	Inter-day		
CBD	2.0	0.9	97	0.61
CBN	0.8	3.5	92	0.65
THC	2.4	2.0	86	4.12
THCA-A	0.2	2.6	97	1.72

Table 2: Precision (relative standard deviation, RSD) and accuracy (RSD) data for all analyte.

The present method was applied to the analysis of different cannabis plant samples. The preparation of these samples was the same as described above. The major constituents of cannabis samples were identified and determined quantitatively, using UPLC/PDA-QDa. Cannabinoid concentrations of these samples of herbal cannabis are listed in Table 3. The THCA-A and THC concentrations varied from 0.16% to 0.99% and from 0.04% to 0.61%, respectively.

Sample	CBD/%	CBN/%	THC/%	THCA-A/%	Total THC*/%
1	0.07	0.01	0.04	0.99	0.91
2	0.04	0.05	0.61	0.48	1.03
3	0.05	0.03	0.49	0.16	0.63

*Total THC=THC+THCA-A×314/358

Table 3: Average concentration of the cannabinoids in different samples by UPLC/PDA-QDa

This method was used to analyze plant samples for potency monitoring. The method permits the determination of the original composition of the cannabinoids in the plant by direct analysis. GC-MS analysis for cannabinoids was performed according to international guidelines for forensic toxicological analysis [11]. The "total THC" concentration comprises the sum of the amount of original THC and the amount which is formed from the THCA-A by decarboxylation during the injection into GC. Results are listed in Table 4. The UPLC method got a higher value of total THC than GC, maybe due to the partial conversion of THCA-A into THC during the measurement of the total THC by GC [12], and degradation of THC will result in CBN [16]. In contrast to practical thermal decarboxylation, the presented method combines quantitation of both analytes, THCA-A and THC without decarboxylation.

Discussion and Conclusions

The presented method for the determination of several cannabinoids in seized cannabis proved to be specific and selective. Different cannabinoids in the chromatogram were baseline-separated. Specificity of the method was further established by analysis of QDa. The method was linear in the respective calibration ranges, with correlation coefficient values over 0.999. The quantitation limits range was from 0.2 µg/mL to 0.4 µg/mL for THC, CBD and CBN, and 2 µg/mL to 40 µg/mL for THCA-A. The validation process showed sufficient precision and accuracy.

The total THC content is calculated as the sum of THC and THCA-A. The conversion factor "THCA-A to THC" takes the different molecular weights into account. The described UPLC-PDA method can be used for monitoring the psychotropic potency of cannabis by quantitation of total THC content (THC and THCA-A), and getting the information of full cannabinoid profiles (acid and neutral cannabinoids).

In conclusion, the presented method for the quantification of THC, THCA-A, CBD, and CBN in cannabis is simple, versatile, flexible, accurate and precise. UPLC coupled with PDA and QDa was used for the simultaneous identification and quantification of the 4 main cannabinoids in cannabis. This analytical method can be used for diverse applications, e.g. plant phenotype determination, evaluation of psychoactive potency and control of medicinal sample quality.

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Sample	UPLC THC+THCA×314/358	GC Total THC	$T_{0.05}$	Comparison of UPLC and GC		Bias/%
				t	Significance	
1	0.91	0.86	2.571	4.36	Yes	3.80
	0.93	0.88				
	0.89	0.88				
	0.92	0.87				
	0.91	0.89				
	0.90	0.88				
2	1.03	0.92		19.12	Yes	11.51
	1.03	0.92				
	1.04	0.92				
	1.01	0.93				
	1.05	0.94				
	1.04	0.93				
3	0.63	0.72		30.74	Yes	-14.29
	0.64	0.75				
	0.63	0.74				
	0.63	0.73				
	0.62	0.73				
	0.63	0.74				

Table 4: Results of UPLC analyses compared to the GC by t-test.

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The analysis of 195 illicit drugs, medicinal substances and their metabolites in blood with liquid chromatography tandem mass spectrometry (LC-MS/MS)

Ms. Lei Weng Tong
Forensic Science Department of Judiciary Police, Macau SAR, P. R. of China
Email: dcf@pj.gov.mo

Abstract

According to the United Nations Office on Drugs and Crime (UNODC), drug-facilitated crime (DFC) refers to criminal acts such as sexual assault, rape, robbery or money extortion which are carried out by means of administering a substance to a person with the purpose to impair the person's behaviours, consciousness, perceptions or decision-making capacity [1]. The substances being used in these cases comprise a broad range of substances, including alcohol, over-the-counter medicines, prescription drugs, illicit drugs and even new psychoactive substances (NPS). Many substances involved in DFC cases are highly potent, effective in single low doses, fast acting and have a relatively short plasma half-life, indicating that their concentration in body fluids may be very low. For drug-facilitated sexual assault (DFSA) cases, it is typical that the victims do not report the incident until several days after the event. This leads to a time delay between the event and the subsequent collection of biological specimen from the victims. To successfully detect DFSA and other DFC related substances, forensic laboratories need to employ highly selective, sensitive and broad-spectrum analytical methods. The present study was aimed to develop a fast, sensitive and comprehensive LC-MS/MS blood qualitative analysis method which incorporates the DFSA related substances as listed on the UNODC DFSA forensic analysis guidelines [1] together with other toxicological relevant substances such as illicit drugs, NPS, pesticides, medicinal substances and their metabolites. The method was validated according to SWGTOX guidelines [2].

Experimental

Sample Preparation

To 0.5 mL of blood, 20 µl of internal standard mix (at 500 ng/ml, see Table 1 for list of internal standards used) was added followed by 1 mL of pH 9 borate buffer. The sample was vortexed briefly and 5 mL of extraction solvent (hexane/ethyl acetate/isoamyl alcohol 2:2:1 v/v) was then added. The sample was centrifuged for 10 minutes at 4000 rpm to separate layers and the organic layer was evaporated to just dry under nitrogen at 50°C. The residue was reconstituted with 200 µL 70:30 deionsed water/acetonitrile and subjected to LC-MS/MS analysis.

Instrumentation

The LC-MS/MS system consisted of an Agilent Technologies 1200 series liquid chromatograph coupled to an AB Sciex QTrap 5500 mass spectrometer operating with TurboIonSpray source. The conditions were as follows:

LC:

- Column: Restek Allure PFP Propyl (5 µm, 50 x 2.1 mm) analytical column at 40°C
- Mobile phase *A:* 2 mM ammonium formate with 0.2% formic acid in deionised water; and mobile phase B: 2 mM ammonium formate with 0.2% formic acid in acetonitrile.
- Gradient: initial isocratic conditions of 30% B, 0.2 mL/min for 3 min; increased to 95% B at 10 min and held for 1 min; flow rate increased to 0.5 mL/min until 13 min and held for 3 min; returned to starting parameters at 16.5 min and held for 3.5 min. Total run time was 20 min.
- Injection volume: 5 µL

MS/MS:

- Curtain gas: 30 psi
- Ion spray voltage: ±4000V
- Ion source temperature: 500°C
- Gas one (nebulizer gas): 40 psi; and gas two (heater gas): 70 psi.
- Scheduled multiple-reaction monitoring (sMRM) acquisition mode with polarity switching; detection window: 90s; target scan time: 2s.

Identification Criteria

1. Analyte retention time should be within 2% of positive control.
2. Signal-to-noise ratio of both MRM transitions should be greater than or equal to 3.
3. MRM ratios to be within ±20% for ratio ≥0.5; ±25% for ratio >0.2-0.5; ±30% for ratio >0.1-0.2 and within ±50% for ratio ≤0.1 of positive control [3].

Results and Discussion

Assay optimization

The developed assay contained 195 substances and 10 deuterated internal standards. MRM transitions were optimized by direct infusion of each analyte in methanol and two pairs of MRM transitions were selected except for methylphenobarbital, for which only one pair of transition ion was monitored due to lack of fragmentation. The retention times and MRM parameters are listed in Table 1.

Assay validation

According to SWGTOX method validation guidelines [2], the validation of a qualitative method involves the evaluation of interferences, ionization suppression/ enhancement, limit of detection and carryover. Carryover was not evaluated in the present study as it would be monitored through quality control practices such as solvent blanks and negative control samples within the analytical procedure.

♦ Interference

By the analysis of ten sources of blank blood samples, zero sample and fortified samples, it was shown that there was no endogenous and exogenous interference from the blank matrix components and deuterated internal standards. It is worth-noting that there were several isomeric pairs included in the assay and those that were not resolved chromatographically were methamphetamine and phentermine, and amobarbital and pentobarbital. Although methamphetamine and phentermine shared the same major transition ion, they could be distinguished by their characteristic fragment ions. Further analysis would be required to distinguish amobarbital and pentobarbital as they produced the same product ions.

♦ Limit of Detection (LOD)

The LOD was determined by fortifying blank blood samples at decreasing concentrations (50 ng/mL down to 0.1 ng/mL) and analysed in three separate runs. The LOD result, as summarised in Table 1 showed that with the exception of pregabalin, gabapentin, pyridostigmine and cyclobarbital, all target analytes have LOD smaller than or equal to 10 ng/mL with approximately 76% of target substances had LOD of ≤1 ng/mL. In addition, the LODs were also well below the therapeutic concentrations (except for neostigmine, clonidine, lormetazepam, perphenazine and flupentixol).

♦ Ionization suppression/ enhancement

Ionization suppression or enhancement was evaluated by the post-extraction addition approach by comparing the area response of the target analytes in unextracted neat samples at 50 ng/mL with the response in extracted blank blood samples from ten different sources which were fortified at 50 ng/mL after extraction. The result, as presented in Table 1, showed that out of the 195 target analytes, about 72% of them experienced average ionization suppression or enhancement of below ±25% while the %CV of the suppression or enhancement was below 15% for about 90% of the analytes. It can be seen that some analytes, such as THC and flupentixol, had potentially significant signal suppression which might have led to elevated detection limits.

♦ Stability

The stability of the blood samples through three freeze-thaw cycles and of processed sample at different storage conditions was evaluated. Out of the 195 target analytes, heroin is the only substance that was unstable in blood as it became undetected in the third freeze-thaw cycle. The target analytes were found to be stable on the auto-sampler at room temperature for 24 hours and in the fridge at 4°C for 30 hours after extraction. It is worthy to note that significant decrease in responses was observed for olanzapine, oxazolam and cloxazolam in processed samples though they still remained detectable under the current conditions.

Conclusion

A sensitive LC-MS/MS method was developed which comprised a comprehensive list of substances commonly encountered in DFSA cases as well as other toxicologically relevant substances. The target analytes ranged from illicit drugs, NPS, pesticides, common over -counter medications, subscribed medicinal substances and their metabolites. A comprehensive validation procedure was performed to demonstrate that this qualitative, multi-analyte method is robust and fit for purpose. If the need arises, the method can be further improved for some analytes that require lower detection limits. The method can also be easily modified to encompass new substances or applied in the analysis of other biological matrices such as urine.

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No	Analyte	MRM ion pair		RT	DP	EP	CE	LOD	Matrix Effect	
		Q1	Q3						%	%CV
1	Amphetamine	136.1	91	3.83	16	8	23	0.5	-10.6	1.9
		136.1	119	3.82	16	8	13			
2	Methamidophos	142.1	112	1.05	56	5.1	16.8	0.5	-23.3	3.6
		142.1	93.9	1.04	56	5.1	19.3			
3	Methamphetamine	150.1	91	4.75	21	10	25	0.5	-11.8	4.2
		150.1	119	4.77	21	10	15			
4	Phentermine	150.1	91	4.65	38	10	27	0.5	-15.4	5.3
		150.1	133	4.65	38	10	14			
5	Norephedrine	152.1	134.1	2.57	18	11.7	16.2	1	-16.4	3.6
		152.1	117.1	2.57	18	11.7	23.5			
6	Paracetamol	152.1	110	1.22	46	5	23	5	-70.0	5.6
		152.1	93	1.22	46	5	29			
7	Amantadine	152.1	135.1	3.93	44	9.7	28.6	1	-13.5	2.0
		152.1	77	3.93	44	9.7	55.6			
8	Pregabalin	160.1	55	2.63	16.5	12.2	32.6	50	1.5	2.0
		160.1	97	2.65	16.5	12.2	20.4			
9	Methomyl	163.1	88	1.74	47	12.1	15	5	-15.5	2.0
		163.1	106	1.74	47	12.1	15			
10	PMA	166.1	121.1	4.17	31.1	11.2	26.3	0.5	-6.4	1.3
		166.1	149.1	4.17	31.1	11.2	13.9			
11	Ephedrine	166.1	148.1	3.14	30	2	15.4	1	-15.4	1.6
		166.1	133.1	3.14	30	2	27			
12	Benzocaine	166.1	138.1	4.91	34	6	15	5	-2.5	4.1
		166.1	94	4.91	34	6	21			
13	Levetiracetam	171.1	126.1	1.07	38	9.3	23.2	0.5	-56.5	8.5
		171.1	154.1	1.07	38	9.3	10.8			
14	Gabapentin	172.1	154.1	2.49	56	9	20.3	50	-7.2	1.3
		172.1	137.1	2.49	56	9	21.6			
15	BZP	177.1	91	4.2	78	9	36	0.5	-15.7	4.6
		177.1	65	4.2	78	9	63			
16	Mephedrone	178.2	160.1	5.26	56	10	19	0.5	-6.2	1.2
		178.2	145.1	5.26	56	10	30			
17	Nikethamide	179.2	108.1	1.94	77.8	12.5	26.8	0.5	-10.1	1.3
		179.2	80	1.94	77.8	12.5	38.6			
18	Phenacetin	180.1	110.1	3.13	64.4	6.2	29.3	0.1	-9.0	1.5
		180.1	138.1	3.13	64.4	6.2	21.5			
19	MDA	180.1	163.1	4.05	26	2	14	0.5	-8.8	2.2
		180.1	135.1	4.05	26	2	26			
20	Memantine	180.2	163.1	9.19	23.4	8.2	21	0.1	-29.2	3.7
		180.2	107.1	9.18	23.4	8.2	35.7			
21	Pyridostigmine	181	72	2.09	50	4	26	50	0.0	1.9
		181	124.1	2.09	50	4	20			

Table 1: MRM transitions parameters, LOD and retention time of target analytes.

No	Analyte	MRM ion pair		RT	DP	EP	CE	LOD	Matrix Effect	
		Q1	Q3						%	%CV
22	Theophylline	181	124	1.06	66	5	25	*	*	*
		181	96	1.06	66	5	33			
23	Acephate	184	143	1.05	61.3	3.1	14	5	-57.8	3.9
		184	113	1.05	61.3	3.1	31.5			
24	MDMA	194.2	163.4	5.04	26	4	17	5	-8.2	1.9
		194.2	135.3	5.05	26	4	29			
25	Caffeine	195.1	138.1	1.25	51	4	27	*	*	*
		195.1	110	1.24	51	4	30			
26	Methylecgonine	200.1	182.1	1.6	51.2	8	24.6	1	-13.7	1.5
		200.1	82.1	1.6	51.2	8	33.7			
27	Dexemdetomidine	201.2	95	9.64	102.1	7.6	27.3	0.5	-19.6	3.4
		201.2	68	9.64	102.1	7.6	47.4			
28	Carbaryl	202.1	145.1	7.7	29	4.2	19.1	0.1	-6.4	2.1
		202.1	117.1	7.7	29	4.2	33.1			
29	Levamisole	205.1	178.1	5.42	70	7.8	28.9	1	-4.0	1.5
		205.1	91	5.42	70	7.8	50.6			
30	Methylone	208.1	160.1	3.8	52	7	26	0.1	-6.4	1.4
		208.1	132.1	3.8	52	7	37			
31	MDEA	208.2	163.1	6.72	42	4	18	0.1	-14.9	1.5
		208.2	133.1	6.72	43	4	26			
32	Varenicline	212.2	169.1	1.9	25.7	3.4	34	0.5	-29.3	3.3
		212.2	183.1	1.9	25.7	3.4	31.4			
33	Meprobamate	219.2	158.2	2.13	48	4	14	0.5	-13.2	3.0
		219.2	97.1	2.13	48	4	21			
34	Neostigmine	223.2	72	3.03	50.9	9.7	41.1	5	-3.1	0.8
		223.2	208.1	3.03	50.9	9.7	28.3			
35	Norketamine	224.1	125.1	4.14	38	3.6	32	0.1	1.8	1.1
		224.1	207.1	4.14	38	3.6	19			
36	Clonidine	230.1	124	4.74	56.2	7.9	57.2	5	-6.1	5.1
		230.1	160	4.74	56.2	7.9	50.7			
37	TFMPP	231.1	188	10.5	68	5	32	0.1	-51.2	6.4
		231.1	118.1	10.5	68	5	56			
38	alpha-PVP	232.2	91	10.5	65.5	8.6	32.4	5	-35.8	6.0
		232.2	77	10.5	65.5	8.6	63.2			
39	Methylphenidate	234.3	84	7.98	38	12	28	0.1	-12.8	2.1
		234.3	56	7.98	38	12	65			

No	Analyte	MRM ion pair		RT	DP	EP	CE	LOD	Matrix Effect	
		Q1	Q3						%	%CV
40	Lidocaine	235.3	86.1	6.84	58	3	29	0.1	-9.2	2.1
		235.3	58.1	6.84	58	3	54.8			
41	Procaine	237.2	100.1	3.89	80	6.2	23	0.1	-3.5	1.3
		237.2	120.1	3.88	80	6.2	35			
42	Carbamazepine	237.2	194.1	4.33	82	8	28	0.1	-4.0	1.5
		237.2	192.1	4.33	82	8	34			
43	Ketamine	238.1	125.1	5.13	70	3.6	40	0.1	-5.3	1.3
		238.1	179.1	5.12	70	3.6	24			
44	Phencyclidine	244.3	91	14.1	32	6	50	0.1	-12.2	10.6
		244.3	86	14.1	32	6	16			
45	Mepivacaine	247.2	98.1	6.05	64	10	23.3	0.1	-6.6	1.2
		247.2	70	6.05	64	10	61.2			
46	Methoxetamine	248.2	203.1	7.59	12	3.9	22.9	0.1	-9.7	2.2
		248.2	121.1	7.58	12	3.9	39.7			
47	Meperidine	248.3	220.2	9.33	75	6	32	0.1	-33.0	3.4
		248.3	174.2	9.33	75	6	31			
48	Methaqualone	251.1	91	7.55	66	5	55	0.1	-11.0	1.9
		251.1	132.1	7.55	66	5	37			
49	Rivastigmine	251.2	206.1	6.51	47.4	11	20.5	0.5	-7.8	0.5
		251.2	86.1	6.51	47.4	11	33.1			
50	Phenytoin	253.2	182	4.97	51	13	25	0.5	8.7	3.3
		253.2	104	4.97	51	13	45			
51	Lamotrigine	256.1	43	5.99	69.1	4	78	0.5	-10.1	1.3
		256.1	211	5.99	69.1	4	36.4			
52	Diphenhydramine	256.2	167.1	14.05	51	7	23	0.1	-11.7	13.4
		256.2	165.2	14.05	51	7	64			
53	Atomoxetine	256.2	44	14.25	39.4	3.9	47.2	0.1	-27.9	18.2
		256.2	148.1	14.25	39.4	3.9	11.6			
54	2C-B	260	243	8.14	51	5	17	5	-11.9	2.3
		260	228	8.14	51	5	29			
55	Phorate	261.1	75	11	34.1	7.1	17	10	-30.1	9.7
		261.1	47	11	34.1	7.1	52.2			
56	Ropinirole	261.2	114.1	5.55	54.2	9	29.4	0.1	-8.2	0.8
		261.2	132.1	5.55	54.2	9	45.2			
57	Carisoprodol	261.3	176.3	6.49	62	6	11	0.5	-5.8	1.9
		261.3	97.1	6.49	62	6	22			

No	Analyte	MRM ion pair		RT	DP	EP	CE	LOD	Matrix Effect	
		Q1	Q3						%	%CV
58	Tramadol	264.1	58	7.77	50	4	57	0.5	-9.7	2.4
		264.1	246.1	7.77	50	4	18			
59	Mirtazapine	266.2	195.1	7.84	42.2	9.8	36.9	0.1	-16.1	3.3
		266.2	72.1	7.85	42.2	9.8	26.6			
60	Desipramine	267.2	72.1	14.35	57.6	11.7	23.4	0.5	-32.3	20.4
		267.2	193.1	14.35	57.6	11.7	57.4			
61	Nordiazepam	271.1	140.1	7.47	62.7	8.7	42.2	0.1	-21.6	3.3
		271.1	165	7.47	62.7	8.7	37.4			
62	Medazepam	271.2	91	13.25	70	12.5	47.6	0.1	-22.4	9.1
		271.2	207.2	13.25	70	12.5	42.9			
63	Doxylamine	271.2	167.1	9.59	48.3	12	45.1	0.5	-29.8	4.7
		271.2	182.1	9.59	48.3	12	26.4			
64	Dextromethorphan	272.3	128	12.1	53	10	86	5	-53.6	7.5
		272.3	171.1	12.05	53	10	54			
65	Chlorpheniramine	275.2	230.1	11.3	46	6	26	0.1	-30.1	7.5
		275.2	167.1	11.3	46	6	55			
66	Ropivacaine	275.2	126	10.1	56.1	12	28.2	0.1	-37.7	7.7
		275.2	84.1	10.1	56.1	12	56.5			
67	MDPV	276.2	126.1	10.7	44	6	38	0.1	-43.4	6.6
		276.2	135.1	10.7	44	6	35			
68	Cyclobenzaprine	276.2	215.1	14.7	79.7	4.2	50.9	0.1	-22.6	12.5
		276.2	231.1	14.7	79.7	4.2	28.4			
69	Venlafaxine	278.2	58	9.48	40	2.8	62	0.5	-36.1	4.8
		278.2	260.2	9.48	40	2.8	17.4			
70	Amitriptyline	278.3	233.1	14.9	56	6	24	0.1	-25.4	12.1
		278.3	91.1	14.9	56	6	34			
71	EDDP	278.3	234.1	15.7	70	6	49.5	0.1	-6.5	5.3
		278.3	249.1	15.7	70	6	31.4			
72	Imipramine	281.3	86.1	14.7	60	9.8	24.8	0.1	-5.0	18.2
		281.3	193.2	14.7	60	9.8	56.6			
73	Nitrazepam	282.2	236.1	6.88	88	12	33	0.1	-31.3	3.1
		282.2	180.1	6.88	88	12	55			
74	7-Aminoflunitrazepam	284.2	135.1	3.16	88	7.1	37.9	0.1	-12.8	1.5
		284.2	227.1	3.16	88	7.1	34.2			
75	Diazepam	285	193.2	8.88	41	5	47	0.5	-48.2	3.0
		285	154.1	8.88	41	5	39			

No	Analyte	MRM ion pair		RT	DP	EP	CE	LOD	Matrix Effect	
		Q1	Q3						%	%CV
76	Promethazine	285.2	86.1	14.4	36	7	23	0.5	-34.9	17.1
		285.2	71	14.4	36	7	62			
77	7-Aminoclonazepam	286.1	121.1	2.36	90	5.1	40.6	0.5	-22.0	1.7
		286.1	222.1	2.36	90	5.1	33.5			
78	Morphine	286.1	165.3	1.86	41	10.5	49	0.5	-27.8	2.6
		286.1	201.2	1.86	41	10.5	34.4			
79	Hydromorphone	286.2	185.1	2.19	83	10.6	41.2	0.5	-22.6	3.4
		286.2	157.1	2.19	83	10.6	55.3			
80	Oxazepam	287.2	241.1	5.18	88	9	30	5	3.2	3.8
		287.2	77	5.18	88	9	80			
81	Tetrazepam	289.2	225.1	9.65	84	12.8	39	0.5	-45.8	3.5
		289.2	253.2	9.65	84	12.8	47			
82	Bupivacaine	289.3	140.1	12.3	58	4	28	0.1	-28.2	8.7
		289.3	84	12.3	58	4	56			
83	Benzoylecgonine	290.2	168.3	2.85	36	6.5	25	0.5	-9.8	1.7
		290.2	105	2.85	36	6.5	41			
84	Atropine	290.2	124.1	4.22	45	7	32	0.1	-10.3	1.5
		290.2	93.1	4.21	45	7	39			
85	Parathion	292.1	236	11.1	68.1	11.9	23.3	5	-60.6	10.5
		292.1	264	11.1	68.1	11.9	13.6			
86	Melitracen	292.2	247.1	15.4	70.1	10.7	25.3	0.1	-13.8	11.7
		292.2	217.1	15.4	70.1	10.7	46.5			
87	Norsertraline	292.2	159	14.6	57.6	12.2	38.9	10	-49.5	25.2
		292.2	275	14.6	57.6	12.2	11.7			
88	Estazolam	295.2	205.1	6.3	82	9.7	55	0.1	-3.2	0.9
		295.2	267	6.3	82	9.7	37			
89	Trimipramine	295.2	100.1	15.1	66	8.8	26.6	0.1	-27.1	14.6
		295.2	58	15.1	66	8.8	67.4			
90	Nimetazepam	296	250.2	8.47	86	9	29	0.1	-34.5	4.4
		296	221.2	8.47	86	9	39			
91	Alimemazine	299.2	100.1	15	66.1	9.8	25.3	0.1	-25.8	12.6
		299.2	58	15	66.1	9.8	61.2			
92	Codeine	300.2	165.3	2.68	55	3	60	0.1	-11.5	2.1
		300.2	199.2	2.68	55	5	40			
93	Chlordiazepoxide	300.2	282	7.38	78	11.7	33	0.1	-16.3	1.4
		300.2	227	7.38	78	11.7	32			

No	Analyte	MRM ion pair		RT	DP	EP	CE	LOD	Matrix Effect	
		Q1	Q3						%	%CV
94	Metoclopramide	300.2	227.1	6.66	56.6	7.5	29	0.1	-4.1	1.3
		300.2	184	6.66	56.6	7.5	46.9			
95	Hydrocodone	300.2	199.1	3.86	44.5	8.4	43	0.1	-9.8	1.5
		300.2	128	3.86	44.5	8.4	81			
96	Clobazam	301.1	259	8.25	126	9.9	27	0.1	2.2	2.9
		301.1	224.1	8.25	126	9.9	42			
97	Oxymorphone	302.1	284	1.97	56.2	11.9	30.1	0.1	-35.2	1.5
		302.1	227	1.97	56.2	11.9	41.4			
98	Dihydrocodeine	302.2	199.2	2.58	72	7	47	0.5	-11.6	1.3
		302.2	201.1	2.58	72	7	39			
99	Trihexyphenidyl	302.2	98.1	14.8	54.8	12	30.8	0.1	-8.1	16.3
		302.2	70.1	14.8	54.8	12	63.7			
100	Fludiazepam	303.1	211	8.78	82	12.8	45	0.1	-18.8	1.7
		303.1	154.1	8.78	82	12.8	38			
101	Scopolamine	304.2	138.1	3.24	49.9	12	32	0.1	-22.3	2.4
		304.2	156.1	3.24	49.9	12	23.4			
102	Cocaine	304.2	182.3	10.15	42	4	26	1	-32.1	7.7
		304.2	150.1	10.15	36	4	34			
103	Sertraline	306.2	159	15.1	55	8.6	38	0.5	-40.2	20.7
		306.2	275	15.1	55	8.6	18			
104	Zaleplon	306.2	236.1	6.14	57.8	4.2	42.8	0.1	-4.1	1.5
		306.2	264.1	6.14	57.8	4.2	27.3			
105	Zolpidem	308.2	235.1	9.7	76	5	47	0.1	-22.1	4.0
		308.2	263.1	9.69	76	5	35			
106	Benztropine	308.2	167.1	14.8	78.3	5	38.7	0.1	7.9	26.6
		308.2	152.1	14.8	78.3	5	80.4			
107	Alprazolam	309.1	205.1	7.77	71	5	57	0.1	-6.0	2.1
		309.1	281	7.77	71	5	33			
108	Pinazepam	309.2	241	9.6	35	4	39	0.1	-21.2	2.8
		309.2	269.2	9.6	35	4	46			
109	Warfarin	309.3	251	9.19	66	10	30	1	-8.8	3.4
		309.3	163.1	9.19	66	10	18			
110	Fluoxetine	310.2	44	14.9	35.9	10.7	64.4	1	-28.4	15.6
		310.2	148	14.9	35.9	10.7	12			
111	Methadone	310.2	265.2	15.6	30	4	17	0.1	-9.5	9.5
		310.2	105.1	15.55	30	4	35			

No	Analyte	MRM ion pair		RT	DP	EP	CE	LOD	Matrix Effect	
		Q1	Q3						%	%CV
112	Thebaine	312.2	58	7.96	51.8	11	37.5	0.1	-17.7	2.4
		312.2	152	7.95	51.8	11	87.3			
113	Nalorphine	312.2	152.1	2.61	86	10	84	0.1	-15.6	2.1
		312.2	201.1	2.61	86	10	37			
114	Biperiden	312.3	98	14.6	42.4	7.9	34.5	0.1	-3.7	19.3
		312.3	70	14.6	42.4	7.9	70.6			
115	Olanzapine	313.2	256.1	14.75	84	9	31	1	-37.8	13.1
		313.2	84.1	14.75	84	9	31			
116	Ethylmorphine	314.2	152.1	3.69	60	10.9	93	0.1	-8.7	2.3
		314.2	165.1	3.69	60	10.9	65			
117	Flunitrazepam	314.2	268.1	8.48	82	11	37	0.1	-22.9	2.8
		314.2	239	8.48	82	11	46			
118	Ranitidine	315.2	176	2.36	45	9	26.4	1	-7.7	1.5
		315.2	130.1	2.36	45	9	34.4			
119	THC	315.2	193.1	11.7	82.1	7.7	29.7	10	-56.1	8.9
		315.2	123	11.7	82.1	7.7	45.2			
120	Clomipramine	315.2	86.1	15.4	68.1	10.9	26.7	0.5	-32.2	13.7
		315.2	58	15.4	68.1	10.9	72.9			
121	Clonazepam	316.1	270	7.64	114	13.2	35	0.1	-14.0	2.4
		316.1	214.2	7.64	114	13.2	52			
122	Oxycodone	316.2	298.2	3.32	48	5	26	0.5	-14.7	1.8
		316.2	241.1	3.31	48	5	41			
123	Bromazepam	316.2	182	3.29	82	8.9	45	0.5	-1.4	1.1
		316.2	209	3.29	82	8.9	35			
124	Chlorpromazine	319.2	86	15.3	55	3	24	0.5	-30.8	15.8
		319.2	246.1	15.3	55	3	32			
125	Lorazepam	321.2	275	5.86	85	8.7	35	0.5	2.9	1.1
		321.2	229.1	5.86	85	8.7	45			
126	Acetylfentanyl	323.2	188.1	11.2	98.4	9.1	34.6	0.1	-23.1	5.5
		323.2	105.1	11.2	98.4	9.1	51.3			
127	LSD	324.2	208.1	9.61	67.4	10	37.4	0.05	-26.1	5.7
		324.2	281.1	9.63	67.4	10	26.1			
128	Alpha-OH-alprazolam	325.2	297.1	4.68	32	10	38.9	0.5	4.5	7.2
		325.2	205.1	4.68	32	10	63			
129	Citalopram	325.2	109	14.1	57	9	33	0.1	-18.9	19.4
		325.2	262.2	14.1	57	9	26			

No	Analyte	MRM ion pair		RT	DP	EP	CE	LOD	Matrix Effect	
		Q1	Q3						%	%CV
130	Prazepam	325.2	271.1	10	72	9.4	33.6	0.1	-26.1	3.9
		325.2	140	10	72	9.4	47.7			
131	Midazolam	326.1	291.1	11.3	51	11	33	0.1	-34.1	5.7
		326.1	249.1	11.3	51	11	43			
132	Clozapine	327.2	270.1	12.7	46	4	31	0.1	-29.0	9.0
		327.2	192.1	12.7	46	4	57			
133	6MAM	328.1	165.3	3.41	56	7	57	0.1	-13.1	2.3
		328.1	211.3	3.41	56	7	33			
134	Naloxone	328.2	310.1	2.75	78.3	12.9	25	0.1	-18.4	2.0
		328.2	212.1	2.75	78.3	12.9	54.5			
135	Oxazolam	329.1	271.1	7.68	58	11.3	29	0.1	-25.8	7.2
		329.1	140	7.68	58	11.3	45.7			
136	AH-7921	329.1	284	14.2	70	9.6	25.3	0.1	-28.6	18.1
		329.1	173	14.2	70	9.6	48.4			
137	Methotrimeprazine	329.2	100.1	15.1	48	11.1	27.2	0.1	-20.9	11.7
		329.2	58.1	15.1	48	11.1	55			
138	Paroxetine	330.1	192.1	14.2	52.3	11.9	29.2	0.1	-34.6	30.4
		330.1	70.1	14.2	52.3	11.9	44.5			
139	Malathion	331.2	127	10.3	42.1	10	19	1	-38.5	12.2
		331.2	285	10.3	42.1	10	11			
140	THC-OH	331.2	313.3	10.4	56	4.7	18.3	5	-26.9	7.5
		331.2	193.1	10.4	56	4.7	38.9			
141	Piroxicam	332.1	78	6.62	42.3	6.6	87	1	-11.0	1.9
		332.1	95	6.62	42.3	6.6	32.4			
142	3-OH-Bromazepam	332.2	287	2.15	98.1	9.2	28.7	5	-6.3	2.7
		332.2	315	2.15	98.1	9.2	21.3			
143	Lormetazepam	335.1	289	8.08	62	10.8	30.5	5	17.5	4.4
		335.1	317.1	8.09	62	10.8	18.8			
144	25C-NBOMe	336.2	121	15.15	58.1	9.8	29.2	0.1	-12.4	15.6
		336.2	91	15.15	58.1	9.8	70.5			
145	Fentanyl	337.2	105	13.8	85	8	55	0.1	-7.4	7.2
		337.2	188.3	13.8	85	8	35			
146	Papaverine	340.1	202.1	9.9	29.8	11.2	39.3	0.1	-30.6	4.3
		340.1	324.1	9.9	29.8	11.2	39.3			
147	Dextropropoxyphene	340.2	58	14.9	52	13	57	0.5	-11.8	12.3
		340.2	266.2	14.9	52	13	12			

No	Analyte	MRM ion pair		RT	DP	EP	CE	LOD	Matrix Effect	
		Q1	Q3						%	%CV
148	Acetylcodeine	342.1	152	7.52	56	5	95	0.1	-2.0	1.8
		342.1	165	7.52	56	5	67			
149	Naltrexone	342.1	324.1	3.36	51.5	8.1	31.9	0.1	-19.1	2.3
		342.1	55	3.36	51.5	8.1	64.7			
150	Sulpiride	342.2	112.1	2.78	57.4	10	33.2	0.1	-7.0	1.3
		342.2	214.1	2.78	57.4	10	47.3			
151	Triazolam	343.1	239.1	7.81	70	8.8	55	0.1	-0.9	3.4
		343.1	315.1	7.8	70	8.8	39			
152	Cloxazolam	349	305	6.52	66	10	36.6	0.5	-38.0	6.7
		349	139.8	6.52	66	10	42			
153	Phenazepam	351	206.1	8.12	73.8	13.1	47.9	0.1	-7.5	2.6
		351	186.1	8.12	73.8	13.1	44.1			
154	Halazepam	353.1	241.1	10.2	66	10.5	58	0.1	-38.0	5.0
		353.1	325.1	10.2	66	10.5	38			
155	Nalbuphine	358.1	340.1	3.56	31.8	9.8	32.6	0.5	-14.5	1.5
		358.1	41	3.56	31.8	9.8	79.1			
156	AM-2201	360.2	155.1	11.5	100	9.2	36.7	0.1	-26.0	4.4
		360.2	127.1	11.5	100	9.2	73			
157	Cinnarizine	369.2	167.1	15.5	37.9	5.9	25.3	0.5	-27.1	13.9
		369.2	152.1	15.5	37.9	5.9	79.4			
158	Heroin	370.2	165.1	7.51	50	6	60	0.5	13.8	7.1
		370.2	268.2	7.51	50	6	43			
159	Trazodone	372.1	176	10.4	16	5	35	0.1	-37.6	9.2
		372.1	148.1	10.4	16	5	45			
160	Hydroxyzine	375.2	201.1	14.25	41.6	11.1	31.5	0.1	-8.7	27.4
		375.2	166.1	14.25	41.6	11.1	61.3			
161	Haloperidol	376.2	123	14.35	62	8	56	0.1	-3.2	28.0
		376.2	165.1	14.35	62	8	33			
162	Remifentanil	377.1	113	9.35	68	12	40	0.1	-8.1	1.9
		377.1	228.1	9.35	68	12	29			
163	Donepezil	380.2	91	11.95	60	8.2	75.2	0.1	-16.6	3.1
		380.2	65	11.9	60	8.2	113.6			
164	Droperidol	380.2	123	10.95	70	5.7	61.2	0.1	-14.5	2.6
		380.2	165.1	10.95	70	5.7	40.3			
165	25B-NBOMe	382.2	121.1	15.2	92.2	8	26.5	0.1	-13.3	13.3
		382.2	91	15.2	92.2	8	74.7			

No	Analyte	MRM ion pair		RT	DP	EP	CE	LOD	Matrix Effect	
		Q1	Q3						%	%CV
166	Quetiapine	384.2	253.1	11.55	93.6	13.9	31.4	0.1	-17.6	7.0
		384.2	221.1	11.55	93.6	13.9	54.2			
167	Flurazepam	388.2	315	12.45	50	12.5	31	0.5	-7.9	6.7
		388.2	317.1	12.45	50	12.5	27			
168	Cetirizine	389.1	201.1	11.8	34.1	10.7	27	0.5	-58.6	7.4
		389.1	166.1	11.8	34.1	10.7	54			
169	Zopiclone	389.2	245	5.43	48.1	6.9	25.2	5	-6.8	2.1
		389.2	217.1	5.42	48.1	6.9	45.7			
170	Brucine	395.2	324	3.54	85	10	43	0.1	-9.1	2.5
		395.2	244	3.54	85	10	47			
171	Zuclopenthixol	401.2	221.1	15.1	26	6	74.6	0.5	-35.9	14.5
		401.2	231	15.1	26	6	61.3			
172	Perphenazine	404.2	171.2	15.1	52	8	32	0.5	-31.7	13.7
		404.2	143.1	15.1	52	8	39			
173	Amlodipine	409.2	238.1	12.5	41.5	8.1	18.9	0.1	4.2	12.1
		409.2	294	12.5	41.5	8.1	16.9			
174	Risperidone	411.2	191.1	12.7	64.3	6.4	37.9	0.1	-5.4	5.9
		411.2	110.1	12.7	64.3	6.4	63.4			
175	Alfentanil	417.2	268.1	11.5	48	6	25	0.1	-10.0	5.0
		417.2	197.1	11.5	48	6	38			
176	25I-NBOMe	428.1	91	15.3	65.6	6.7	75.6	0.1	-8.0	18.2
		428.2	121.1	15.3	65.6	6.7	30.9			
177	Flupentixol	435.2	305	15.55	78.1	8.2	42.8	5	-51.7	17.4
		435.2	221.1	15.55	78.1	8.2	88.9			
178	Aripiprazole	448.2	285.1	14.15	26.3	6.1	39.5	0.5	-22.0	24.1
		448.2	176.1	14.15	26.3	6.1	45.4			
179	Buprenorphine	468.3	396.2	14	76	6	54	0.1	-13.0	14.3
		468.3	414.3	14	76	6	47			
180	Sildenafil	475.2	58	9.99	100.7	7.4	81.4	0.5	-9.5	3.6
		475.2	100.1	9.99	120	10	47			
181	Barbital	183	42	1.56	-36	-10	-34	5	-2.9	1.4
		183	140	1.55	-36	-10	-17.5			
182	Allobarbital	207	42	2.27	-34	-6	-50.5	5	5.7	7.1
		207	164.1	2.27	-34	-6	-14.4			
183	Aprobarbital	209	42	2.51	-34	-10.3	-40	5	1.7	2.4
		209	166.1	2.5	-34	-10.3	-16.5			
184	Butobarbital	211	42	3.14	-73	-9	-56	5	1.7	4.4
		211	168	3.14	-73	-9	-17.5			
185	Butalbital	223	42	3.53	-26	-4	-44	5	6.1	2.1
		223	180	3.53	-26	-4	-17.5			

No	Analyte	MRM ion pair		RT	DP	EP	CE	LOD	Matrix Effect	
		Q1	Q3						%	%CV
186	Pentobarbital	225	42	4.61	-64	-9.2	-61	5	6.6	3.4
		225	182.1	4.62	-64	-9.2	-18.8			
187	Amobarbital	225	42	4.61	-60	-10.6	-61	5	6.1	3.8
		225	182.1	4.62	-60	-10.6	-18.8			
188	Phenobarbital	231	42	2.78	-26	-8.9	-46	5	4.4	5.0
		231	188	2.78	-26	-8.9	-15.4			
189	Cyclobarbital	235	42	2.97	-51	-6.9	-52.6	50	-0.2	2.4
		235	192	2.97	-51	-6.9	-17.2			
190	Secobarbital	237	42	5.86	-54	-13.2	-49	5	1.2	2.7
		237	194	5.86	-54	-13.2	-17.8			
191	Thiopental	241.1	57.9	7.91	-57	-7	-38.4	5	12.5	4.9
		241.1	100.9	7.9	-57	-7	-20			
192	Methylphenobarbital	245	42	6.03	-28	-7.1	-41	5	0.4	3.1
193	Diclofenac	294.1	250.1	9.74	-50.5	-4.9	-17.7	5	15.1	3.7
		294.1	214.1	9.78	-50.5	-4.9	-26.8			
194	Topiramate	338.1	78	4.25	-77.7	-6.9	-51.7	5	0.3	4.7
		338.1	95.9	4.24	-77.7	-6.9	-30			
195	THC-COOH	343.2	299.3	10.4	-150	-11.1	-29.4	0.5	-24.5	4.3
		343.2	245.3	10.4	-150	-11.1	-38.1			
ISTD1	Amphetamine-d6	142	93.1	3.82	30	5	25	---	---	---
		142	125.2	3.81	30	5	13			
ISTD2	Methamphetamine-d9	159.1	93	4.74	48	4	25	---	---	---
		159.1	125.2	4.74	48	4	16			
ISTD3	Ketamine-d4	242	129.1	5.09	54	9	40	---	---	---
		242	129.1	5.09	54	9	40			
ISTD4	Morphine-d3	289.1	165	1.85	61	5	57	---	---	---
		289.1	151.9	1.85	61	5	53			
ISTD5	Diazepam-d5	290.1	198.1	8.86	41	5	45	---	---	---
		290.1	154.1	8.86	41	5	37			
ISTD6	Cocaine-d3	307.1	185.1	10.2	36	5	27	---	---	---
		307.1	153	10.2	36	5	37			
ISTD7	Alprazolam-d5	314.1	210.1	7.74	71	5	55	---	---	---
		314.1	286	7.74	71	5	35			
ISTD8	6MAM-d3	331	164.9	3.4	91	5	57	---	---	---
		331	211	3.4	91	5	37			

No	Analyte	MRM ion pair		RT	DP	EP	CE	LOD	Matrix Effect	
		Q1	Q3						%	%CV
186	Pentobarbital	225	42	4.61	-64	-9.2	-61	5	6.6	3.4
		225	182.1	4.62	-64	-9.2	-18.8			
187	Amobarbital	225	42	4.61	-60	-10.6	-61	5	6.1	3.8
		225	182.1	4.62	-60	-10.6	-18.8			
188	Phenobarbital	231	42	2.78	-26	-8.9	-46	5	4.4	5.0
		231	188	2.78	-26	-8.9	-15.4			
189	Cyclobarbital	235	42	2.97	-51	-6.9	-52.6	50	-0.2	2.4
		235	192	2.97	-51	-6.9	-17.2			
190	Secobarbital	237	42	5.86	-54	-13.2	-49	5	1.2	2.7
		237	194	5.86	-54	-13.2	-17.8			
191	Thiopental	241.1	57.9	7.91	-57	-7	-38.4	5	12.5	4.9
		241.1	100.9	7.9	-57	-7	-20			
192	Methylphenobarbital	245	42	6.03	-28	-7.1	-41	5	0.4	3.1
193	Diclofenac	294.1	250.1	9.74	-50.5	-4.9	-17.7	5	15.1	3.7
		294.1	214.1	9.78	-50.5	-4.9	-26.8			
194	Topiramate	338.1	78	4.25	-77.7	-6.9	-51.7	5	0.3	4.7
		338.1	95.9	4.24	-77.7	-6.9	-30			
195	THC-COOH	343.2	299.3	10.4	-150	-11.1	-29.4	0.5	-24.5	4.3
		343.2	245.3	10.4	-150	-11.1	-38.1			
ISTD1	Amphetamine-d6	142	93.1	3.82	30	5	25	---	---	---
		142	125.2	3.81	30	5	13			
ISTD2	Methamphetamine-d9	159.1	93	4.74	48	4	25	---	---	---
		159.1	125.2	4.74	48	4	16			
ISTD3	Ketamine-d4	242	129.1	5.09	54	9	40	---	---	---
		242	129.1	5.09	54	9	40			
ISTD4	Morphine-d3	289.1	165	1.85	61	5	57	---	---	---
		289.1	151.9	1.85	61	5	53			
ISTD5	Diazepam-d5	290.1	198.1	8.86	41	5	45	---	---	---
		290.1	154.1	8.86	41	5	37			
ISTD6	Cocaine-d3	307.1	185.1	10.2	36	5	27	---	---	---
		307.1	153	10.2	36	5	37			
ISTD7	Alprazolam-d5	314.1	210.1	7.74	71	5	55	---	---	---
		314.1	286	7.74	71	5	35			
ISTD8	6MAM-d3	331	164.9	3.4	91	5	57	---	---	---
		331	211	3.4	91	5	37			

No	Analyte	MRM ion pair		RT	DP	EP	CE	LOD	Matrix Effect	
		Q1	Q3						%	%CV
ISTD9	Pentobarbital-d5	230.2	42.1	4.44	-54	-3.9	-33.8	---	---	---
		230.2	187	4.43	-54	-3.9	-18.4			
ISTD10	Phenobarbital-d5	236	42	2.75	-45.9	-5.2	-46	---	---	---

Note: RT=retention time (min); DP=Declustering Potential (V); EP=Entrance Potential (V); CE=Collision Energy (eV); LOD=limit of detection (ng/mL); CV=coefficient of variation (%).

* Caffeine-free and theophylline-free blank matrices were not available so their corresponding LOD and ionization suppression/ enhancement were not determined.

A Case Solved by Trace DNA Typing in Mongolia

Mr. Tenuun.B, Mrs. Purevdulam.Sh, Ms. Gantuya.B, Mr. Ganbold.S
National Institute of Forensic Science, Mongolia

Introduction

The case was the first to apply trace DNA analysis evidence to a criminal investigation. During the investigation, DNA profiling not only identified the true perpetrator but it also excluded an innocent suspect. This case demonstrated DNA profiling's great potential in forensic investigations.

Since forensic DNA analysis laboratory established in National Institute of Forensic Science, Mongolia, any cases of crime such as murder, sexual assault and paternity had been solved by DNA testing. In 2017, cases solved by DNA analysis had reached approximately 1200 cases.

In this case study, we present a case solved by trace DNA analysis.

Case

In the eastern region of Mongolia and spring of 2015, a woman who worked in a gas station was robbed and beaten up by three strangers. Over a period of one year, the DNA of twenty suspecting individuals were tested against the evidence DNA. The only key evidence item was a piece of cloth used to mask their faces found at the crime scene (Figure 1). The area of the mask in contact with the mouth were cut up in to 1.5ml tubes for DNA testing.

Materials and Methods

DNA extraction

There was a cloth in the trace evidence where the robbers had been used as the mask (Fig 1). First, when the used "SaLigAE" test the negative result, but we have been extracted DNA. The modified manual method follows this procedure: A 0.5 cm^2 sample of the cloth from fig 1, B side was taken for the extraction. The sample cloth washed in water gently and the solution was centrifuged. The DNA extraction was performed manually using Chelex®100 resin DNA extraction kit (Bio-Rad) using workstation. Supernatant was removed then 200 µl Chelex®100 resin buffer and 0.3 µl Proteinase K was added to remaining solution and mixed thoroughly. The tube was incubated at 56°C for 1 h, then at 96°C for 8 min. After that, sample was centrifuged at 14000 RPM for 5 min and PCR was performed directly from this sample. When the using Nanodrop-1000, DNA quantification was 15 ng/ul.

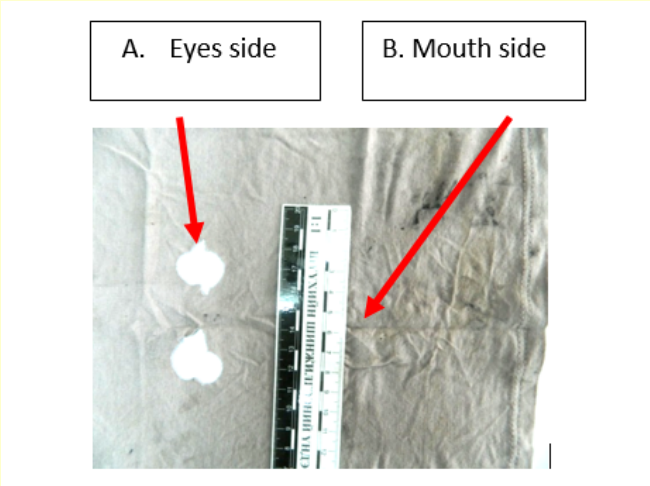

Figure 1. A piece of cloth used by hide their face found at the crime scene.

STR typing

PowerPlex® 16 HS System (Promega) STR typing was carried out following manufacturer's guidelines. The template DNA was diluted to 0.5 ng/µl respectively and amplified 22 cycles by GeneAmp® PCR Systems 9700 thermal cycler. 1 µl PCR products were denatured in 10 µl of loading buffer composed of 9.8 µl HI-DI™ formamide (Applied Biosystems, USA) and 0.3 µl ILS™-600 size standard mixture (PowerPlexHS16, Promega, USA).

STR fragments was separated by capillary electrophoresis using an ABI 3130 Genetic Analyzer (Applied Biosystems, USA). The peak detection threshold was set at 50 relative fluorescence units (RFU), and data analysis was carried out using GeneMapper™ (Applied Biosystems, USA) software.

Result

In this case, we were cut more likely to be in contact with lips from the mask/piece of cloth. Either no DNA was detected or only the suspect's DNA STR were obtained from it. A male STR profile was detected only at the mouth part of the mask. The 15 STR profile obtained were compared with 20 suspects and it only matched one person.

Statistical analysis

Statistical probability of the match was analyzed by Random Match Probability using allele frequencies from the Mongolian Population.

The allele frequencies for the Mongolian Population used for this calculation were previously reported (T.Minjuur, 2008).

№	Loci	Evidence STR profile	Genotype frequency
1	*D3S1358*	15, 16	0.2419
2	*THO1*	7, 8	0.0679
3	*D21S11*	28, 30	0.0264
4	*D18S51*	14, 21	0.0114
5	*PENTA E*	10, 17	0.0156
6	*D5S818*	10, 13	0.0327
7	*D13S317*	12, 12	0.0380
8	*D7S820*	8, 11	0.1525
9	*D16S539*	10, 11	0.0502
10	*CSF1PO*	12, 12	0.1202
11	*PENTA D*	10, 10	0.0148
12	*vWA*	16, 19	0.0350
13	*D8S1179*	11, 16	0.0089
14	*TPOX*	8, 12	0.0399
15	*FGA*	23, 26	0.0217
16	*Amel*	X, Y	

Table 1. DNA STR profile obtained from the mask/piece of cloth were found at the crime scene.

Random match probability was calculated following formula homozygote allelic frequency by p^2 and heterozygote allelic frequency by 2pq.

The genotype found on the mask/piece of cloth retrieved from the gas station, matched with the suspect's genotype with an estimated probability of 1 in 2.84×10^{21}.

Discussion

The male DNA profile obtained from mask/piece of cloth, determined via PowerPlexHS-16 analysis, showed statistical probability of 1 in 2.84×10^{21} in a random man.

In Mongolia previous crime was mainly solved by finger print and other investigation, this was the first robbery case solved by only trace DNA analysis.

We had previously analyzed to using postage stamps, latex glove, caps etc. for them determinate DNA testing identified. In this case, it was very important for the case using trace DNA analysis.

This proved our laboratory's capability for trace DNA analysis.

References

T.Minjuur. Mongolian population study of autosomal short tandem repeat loci, dissertation for Ph.D degree. Ulaanbaatar, Mongolia, 2008.

AFSN Member Institutes

Country/Region	*No.*	*Name of Member Institute (as at March 2019)*
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	Department of Police Medicine of the Indonesian National Police
	6	Eijkman Institute for Molecular Biology
	7	Forensic Laboratory Centre of Indonesian National Police Headquarters
	8	Indonesian Association of Forensic Pathologist
	9	Laboratory of National Narcotics Board
Lao People's Democratic Republic	10	Food and Drug Quality Control Center
Malaysia	11	CyberSecurity Malaysia
	12	Department of Chemistry
	13	Malaysian Communications and Multimedia Commission
	14	Royal Malaysia Police Forensic Laboratory
Mongolia	15	Mongolian National Institute of Forensic Science
People's Republic of China	16	Forensic Science Center of Guangdong Provincial Public Security Department
	17	Forensic Science Division, Department of Fujian Provincial Public Security
	18	Guangzhou Forensic Science Institute
	19	Institute of Forensic Science, Ministry of Public Security
	20	Institute of Forensic Science, Dezhou Public Security Bureau
	21	Institute of Forensic Science, Hangzhou Public Security Department
	22	Institute of Forensic Science, Shandong Public Security Department
	23	Institute of Forensic Science, Suzhou Public Security Bureau
	24	Institute of Forensic Science, Tianjin Public Security Bureau
	25	The Institute of Evidence Law and Forensic Science, China University of Political Science and Law
	26	Forensic Science Division of the Government Laboratory, Hong Kong SAR
	27	Forensic Science Department of Judiciary Police, Macau SAR
Philippines	28	Laboratory Service, Philippine Drug Enforcement Agency
	29	National Bureau of Investigation
	30	National Reference Laboratory for Environmental and Occupational Health, Toxicology and Micronutrient Assay, East Avenue Medical Center, Department of Health
	31	Natural Sciences Research Institute, University of the Philippines Diliman Quezon City
	32	Philippine National Police
Republic of Kazakhstan	33	Forensic Examinations Centre of the Ministry of Justice
Republic of Korea	34	Daejeon Health Institute of Technology, Daejeon Health Sciences University
	35	Graduate School of Forensic Science, Soon Chun Hyang University
	36	Korea Coast Guard Research Institute
	37	National Digital Forensic Center of Supreme Prosecutors' Office
	38	National Forensic Service
	39	Scientific Investigation Center of Korean National Police Agency
	40	Scientific Investigation Laboratory, Ministry of National Defense
Singapore	41	Health Sciences Authority
Sri Lanka	42	Government Analyst's Department
	43	National Dangerous Drugs Control Board
Thailand	44	Central Institute of Forensic Science, Ministry of Justice
	45	Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University
	46	Department of Forensic Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University
	47	Department of Medical Sciences
	48	Faculty of Medicine, Chiang Mai University
	49	Human Genetics Unit, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital
	50	Institute of Forensic Medicine, Police General Hospital, The Royal Thai Police
	51	Narcotics Analysis and Technical Service Institute, Office of Narcotics Control Board
The Republic of the Union of Myanmar	52	Defence Services Medical Research Centre
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	55	National Institute of Forensic Medicine
	56	Forensic Science Institute Vietnam