ForensicAsia

THE ASIAN FORENSIC SCIENCES NETWORK NEWSLETTER | ISSUE 5 | 2013

AFSN President's Address

Congratulations to Dr. Khunying Porntip Rojanasunan of the Central Institute of Forensic Science, Ministry of Justice, Thailand, Chairperson of the AFSN 4th Annual Meeting and Symposium, and her team for successfully organising the eventful symposium on 25th to 28th November 2012 in Bangkok, Thailand. More than 400 participants attended the four-day programme with distinguished speakers from around the world. Besides the DNA, Illicit Drugs, Toxicology, Trace Evidence workgroups and the Quality Assurance & Standard Committee, a new workgroup known as Crime Scene Investigation Workgroup was formed during the annual meeting in Bangkok. Headed by Mr. Ge Baichuan of the Institute of Forensic Science, Ministry of Public Security, People's Republic of China, the new workgroup serves as a vital platform for the regional scientists to discuss ideas, share information, experience and enhance the quality of crime scene investigation.

As we are quickly approaching the 5th AFSN Annual Meeting and Symposium to be hosted by the Health Sciences Authority of Singapore from 11th to 14th November 2013 at the Resorts World Sentosa Convention Centre, Singapore, we are pleased that this annual event of AFSN is beginning to emerge as a major forensic science forum in Asia with active participation by forensic scientists from all over the world.

2013 is the election year for six new Board Members, with the exception of the vice-president represented by Dr. Joongseok Seo of National Forensic Service, Korea, who will take over as President of AFSN for the next term of 2013 – 2015. Please look out for the announcement regarding the nomination of board members, and I encourage all member institutes to participate in the nomination and those elected during the coming Annual Meeting in Singapore shall represent AFSN for the new term 2013 – 2015.

Just to inform that this year's International Forensic Strategic Alliance (IFSA) Annual Meeting was held in Lyon, France, from the 8th to 10th October 2013 in conjunction with the INTERPOL 17th International Forensic Science Managers Symposium. Dr. Angeline Yap and I represented AFSN during the Annual Meeting.

I look forward to see all of you in the exciting city of Singapore in November 2013.

Mr. Lim Kong Boon
AFSN President

AFSN News

The Birth of AFSN CSIWG

Dr. Fu Huanzhang
Institute of Forensic Science, Ministry of Public Security, China

Introduction

Crime Scene Investigation (herein referred to as "CSI") is to search for evidence left behind at a crime scene, including the location, object, body, etc, using scientific and technological methods. The main role of CSI is to recognise, collect and preserve the physical evidence and any related information so as to determine the nature of the case, to analyse the criminal process and to determine the direction and scope of the investigation, thereby better serving the following investigations, prosecution and trial activities. As CSI is the beginning of the investigative work, its quality and level will directly affect the investigation of the whole case.

Owing to the importance of CSI, many international forensic organisations have recently established their own CSI workgroups, desiring to promote the development of CSI. In order to facilitate the communication among the crime scene investigators and to promote the development and standardisation of CSI in Asia, the Asian Forensic Sciences Network (herein referred to as "AFSN") decided to establish its 5th workgroup on CSI in May 2012. Our institute has been nominated by the Board of AFSN to take charge of its establishment.

The CSI Workgroup

A preparatory group within our institute was initially set up to conduct research on AFSN and other similar international organisations. Based on this report and the actual situation of CSI in Asia, the group had drafted a series of documents, such as Proposal of Establishment, Execution Plan, Terms of Reference, with assistance from the Secretariat of AFSN. Finally, these documents were submitted to the Board and received approval at the 4th AFSN Annual Meeting held in Thailand in November 2012. This marked the formal establishment of CSIWG.

As a scientific workgroup of AFSN, CSIWG is dedicated to improve the ability and level of crime scene investigation in Asia by strengthening the communication, exchanges, discussion and cooperation among AFSN member institutes and other organisations in the domain of crime scene investigation, as well as with their crime scene investigators. Its main goals can be concluded as: (1) To promote the development of crime scene investigation in Asia; (2) To enhance the standardisation and modernisation of the techniques used in crime scene investigation; (3) To enhance the training in crime scene investigation; (4) To improve the level of quality management in crime scene investigation; (5) To promote the technical exchange and cooperation in crime scene investigation; (6) To provide a platform for information exchange and sharing.

Establishment of CSIWG at the 4th AFSN Annual Meeting in Bangkok, Thailand, in November 2012.

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Editor's Address

Dear readers,

2013 is indeed an exciting year as we look forward to the 5th AFSN Annual Meeting and Symposium which is to be hosted by Health Sciences Authority (HSA) in Singapore. There has been a change to the editorial committee in this issue. We would like to record our appreciation to Dr. Angeline Yap (Founding Editor) for her invaluable contributions to the past issues. She has been the driving force of ForensicAsia. We look forward to her continued input as Editorial Advisor. I would also like to take this opportunity to express my thanks and gratitude to all the Editorial Committee members for all the support and assistance rendered in reviewing the articles.

Five years have passed since the inaugural issue of Forensic Asia was published in 2009. As we bring with us the memories from the 4th ASFN Annual Meeting and Symposium in Bangkok, Thailand, we are reminded of how far and wide our forensic network has grown. Likewise, as the newsletter comes to its fifth year, we are also receiving more quality submissions featuring interesting case studies from the various forensic disciplines as well as technical articles describing novel methods. This issue of the newsletter is a new bumper edition with 11 technical articles and 7 case studies. There is also a change in appearance as we adopt a journal layout for the scientific articles. Please do give us your feedback and comments at hsa_asg@hsa.gov.sg.

We hope that all of you will enjoy reading the interesting articles in this issue and we look forward to seeing even more submissions for the next issue!

Dr. Christopher Syn
Editor

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.

The Current Status and Future Strategy of the Automated Fingerprint Identification System in China

Dr. Ma Rong Liang
Institute of Forensic Science, Ministry of Public Security, China

The Automated Fingerprint Identification System (AFIS) is one of most important scientific approaches for criminal investigation and has been widely used in policing in China. Currently, there are about 110 million tenprints and 2 million latents in the database of the Chinese Police. In 2011-2012, over 200,000 criminal cases were solved using AFIS at the provincial level and over 20,000 criminal cases have been uncovered by cross-provincial cooperation with AFIS in China.

It should be noted that AFIS in China was built on the provincial level and there is no National Automated Fingerprint Identification System (NAFIS). Now, there are six types of AFIS from different manufacturers throughout the 31 provinces of mainland China. The AFIS enables fingerprint searches freely within each province, but the cross-provincial searches are not yet available.

In order to enable cross-provincial fingerprint searches, the Ministry of Public Security (MPS) established an Assistant Fingerprint Search Platform (AFSP) in 2005. In this system, the Provincial Police forwards the search request to the AFSP and the AFSP personnel will distribute the search task to all the other provinces. The search would then be done in other provinces and the results returned to the AFSP at different times, according to the seriousness of the crimes. Finally, the AFSP personnel will have the statistics done on all the results from cross-provincial searches and provide feedback to the original province.

However, the differences between AFIS produced by different manufacturers pose a significant challenge to the in-depth application of AFIS. To resolve this problem, the MPS initiated the accreditation of the provincial AFIS. The aim of accreditation is to strengthen the cooperation in criminal investigation using AFIS from different manufacturers, thus eliminating the barriers of different software and hardware and finally improving the communicating of information across different AFIS. The MPS promulgated a series of standards that all the provincial AFIS have to comply with. For example, all the AFIS are required to have input ports that permit the accredited livescan system to transfer fingerprint data, and must have ports connected to the AFSP that can be jointly operated by other provinces. Only after the above requirements are met would the AFIS then have passed the accreditation. The AFIS that fails this accreditation will be disallowed from use within the Chinese Police. The accreditation is divided into two stages. The first stage is the software accreditation in the laboratory, which has been completed. The second stage is the on-site test in local police facilities that will be completed in 2013.

The AFSP and the accreditation for AFIS can partially solve the problems in the current Chinese AFIS, but there still remains the strong need to establish a NAFIS for China. There should be more than 100 million tenprints and more than 10 million latents in the database of the future Chinese NAFIS. Although an enormous AFIS will bring about challenges of high costs and other difficulties that would not be faced by a smaller database, it will benefit the Chinese Police for crime investigation in the long run. Investigations are currently underway for this project.

The European DNA Profiling Group – EDNAP

Professor Niels Morling
Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Introduction

The European DNA Profiling (EDNAP) group was established in 1988 by forensic genetic scientists from European countries. The initial purpose of EDNAP was to harmonise DNA technologies for crime case investigations so that DNA results could be exchanged across borders in Europe. In 1991, EDNAP was included among the working groups of the ISFG. EDNAP consists of approximately 20 European laboratories. The EDNAP group collaborates closely with the DNA Working Group of the European Network of Forensic Science Institutes - ENFSI. EDNAP organises exercises in order to explore the possibility of standardisation of new forensic genetic methods. For more information, please visit http://www.isfg.org/EDNAP.

EDNAP has no statutes. All decisions are made unanimously. EDNAP has 20 member laboratories and they can be found listed at http://www.isfg.org/EDNAP/Members. The secretary of EDNAP (http://retsmedicin.ku.dk/english/aboutnew/directorofdept/) coordinates the work of the group. The list of secretaries of EDNAP is as follows:

- 1996 – Now: Professor Dr. Niels Morling, Copenhagen
- 1988 – 1996: Dr Peter Martin, London

EDNAP Meetings

EDNAP meets once every 6 months. The meetings are, where possible, held in conjunction with meetings of the DNA Working Group of the European Network of Forensic Science Institutes (http://www.enfsi.eu/about-enfsi/structure/working-groups/dna). Guests from other parts of the world regularly attend the meetings. More information can be found at http://www.isfg.org/EDNAP/Meetings.

Collaborative EDNAP exercises

The most important objective of EDNAP is to explore new forensic genetic methods. This is done through collaborative exercises among EDNAP laboratories and other interested parties. More than 25 reports have since been published. Please visit http://www.isfg.org/EDNAP/Publications from where the publications can be downloaded.

Introduction of Legislation for the Control of New Psychoactive Substances in Singapore

Dr. Ong Mei Ching, Ms. Wendy Lim Jong Lee, Dr. Angeline Yap Tiong Whei
Health Sciences Authority, Singapore

Introduction

New psychoactive substances (NPS) refer to substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat [1-3]. Examples of NPS include synthetic compounds such as synthetic cannabinoids and synthetic cathinones, and psychoactive components of plants such as kratom and *Salvia divinorum*.

Figure 1 shows the types of NPS and related controlled drugs encountered in our laboratory in the period January 2011 to February 2013. Among the drugs shown in Figure 1, those highlighted in red are Class 'A' controlled drugs in the Misuse of Drugs Act of Singapore. They include 4-MMC, MDA, MDMA, ketamine, TFMPP, 1-BZP and 2C-B. (Note: The data shown for amphetamines are those which occurred together with other NPS.)

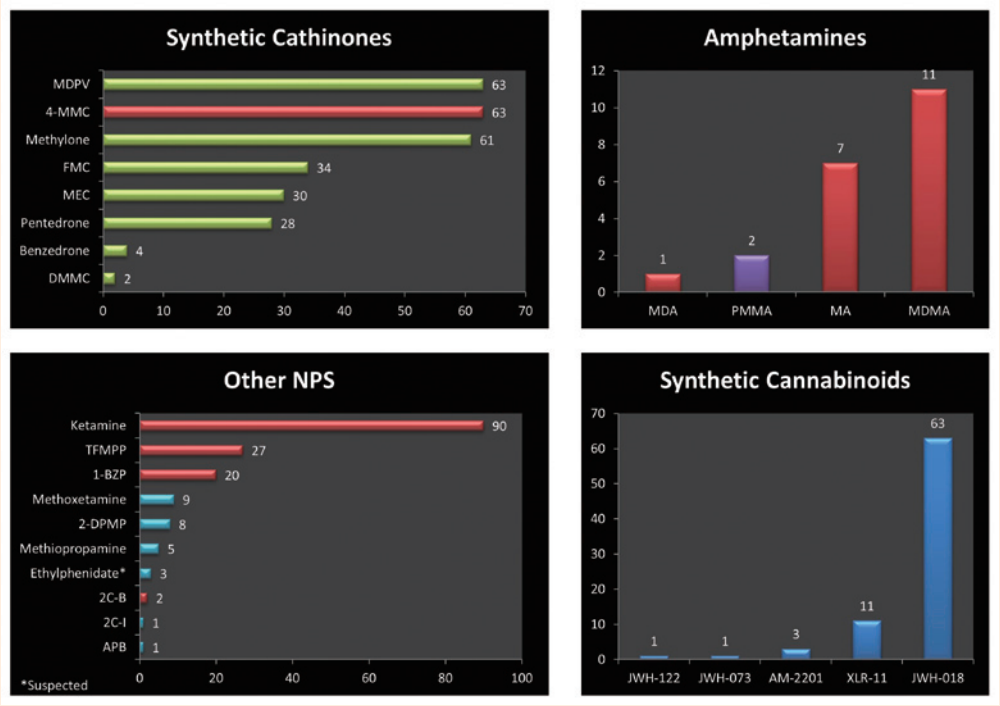


Figure 1: Occurrences of NPS and related controlled drugs from January 2011 – February 2013.

In recent years, NPS have frequently been encountered in Ecstasy-type tablets in our laboratory. These NPS, when present in Ecstasy tablets, are usually present in multiple components. Figure 2 shows two examples of Ecstasy-type tablets our laboratory had received. The tablet stamped with an imprint of a four-leaf clover was found to contain ketamine, 1-BZP, MEC, 4-MMC, TFMPP, MDPV, 1,4-DBZP and JWH-018. While the tablet stamped with an imprint of a kangaroo was found to contain FMC, MEC, DMMC, methylone, ketamine, MDPV, JWH-018, AM-2201, 1-BZP and TFMPP.

Besides Ecstasy-type tablets, synthetic cannabinoids have also been detected in vegetable matter. Other NPS such as methoxetamine and methiopropamine have been encountered in the form of a white powdery substance. Mitragynine and Salvinorin A, which are psychoactive components of the kratom and *Salvia divinorum* plants respectively, have been detected in plant materials.

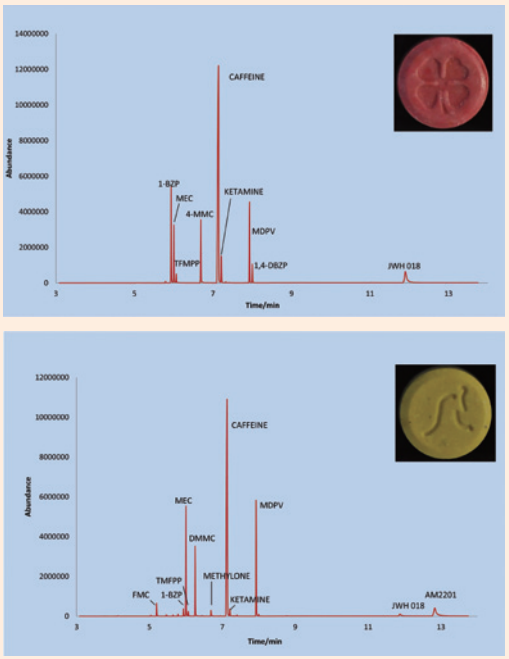


Figure 2: Ecstasy tablets with 'four-leaf clover' and 'kangaroo' imprints

Legislative control of NPS

With the proliferation and abuse of NPS, many countries have started placing these substances under legislative control. Some countries have incorporated these substances into the permanent schedules of controlled drugs/substances, while others have placed them under temporary control, pending review after a certain time period.

In Singapore, as of 1 May 2013, legislative amendments were enacted to the Misuse of Drugs Act (CAP. 185) [4]. These include the listing of NPS into a temporary schedule that empowers the enforcement officers to seize and destroy these substances. This will restrict the circulation of these NPS while relevant scientific analysis and industrial consultations are being carried out. The list of NPS in the temporary schedule will be periodically reviewed.

The temporary schedule (hereafter referred to as the Fifth Schedule), consists of eleven paragraphs. Ten of these paragraphs are the generic classifications of synthetic cannabinoids and synthetic cathinones. In this case, the substances which are controlled under the different generic classes are defined by specifying a certain core molecular structure as well as substituents on the molecule. Generic legislative control allows a large number of compounds to be encompassed under the definitions [5]. This is advantageous in the case of synthetic cannabinoids and cathinones as simple modifications are often made to a similar class or type of compounds. For other NPS which do not fall under the ten generic classes, they are listed individually in a separate paragraph.

The eight generic classes of synthetic cannabinoids are the following: naphthoylindoles, naphthylmethylindoles, naphthoylpyrroles, naphthylideneindenes, phenylacetylindoles, cyclohexylphenols, benzoylindoles and adamantoylindoles. The general structures are shown in Figure 3, where the specific substituents (R group) refer to the following: an alkyl, haloalkyl, alkenyl, cycloalkylmethyl, cycloalkylethyl, 1-(N-methyl-2-piperidinyl)methyl or 2-(4-morpholinyl)ethyl group. An example of the generic definition for naphthoylindoles in the Fifth Schedule is as follows:

"Any compound containing a 3-(1-naphthoyl)indole structure with substitution at the nitrogen atom of the indole ring by an alkyl, haloalkyl, alkenyl, cycloalkylmethyl, cycloalkylethyl, 1-(N-methyl-2-piperidinyl)methyl or 2-(4-morpholinyl)ethyl group, and any derivatives of the above compounds containing hydroxy and/or carboxylic acid groups, whether or not further substituted in the indole ring to any extent and whether or not substituted in the naphthyl ring to any extent, including any salt or stereoisomeric form of the above compounds or derivatives, and any preparation or product containing the above compounds or derivatives."

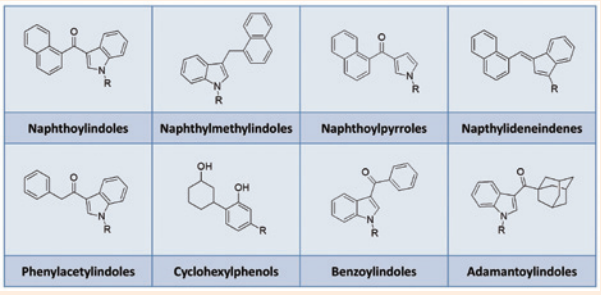


Figure 3: Eight generic classes of synthetic cannabinoids.

Paragraphs 9 and 10 are the generic definitions of synthetic cathinones and pyrovalerones, which are a sub-class of cathinones.

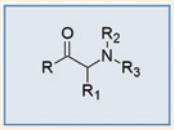


Figure 4: General structure for synthetic cathinones and pyrovalerones.

Synthetic cathinones and pyrovalerones have the basic structure 2-aminopropan-1-one (Figure 4). Synthetic cathinones which fall under the generic definition of paragraph 9 include the following:

(a) R = phenyl ring, including substitution in the phenyl ring to any extent with alkyl, alkoxy, alkylenedioxy, haloalkyl, or halide substituents, whether or not further substituted in the phenyl ring by one or more other univalent substituents;
(b) R1 = an alkyl substituent;
(c) R2/R3 = substitution at the nitrogen atom with alkyl or dialkyl, benzyl or methoxybenzyl groups, or by inclusion of the nitrogen atom in a cyclic structure.

For synthetic pyrovalerones which fall under the generic definition of paragraph 10, the R-group can be any monocyclic, or fused-polycyclic ring system, including any substitutions to the ring system. The ring system excludes a phenyl ring or alkylenedioxyphenyl ring system, which are already defined in paragraph 9. Other modifications (i.e. R1, R2 and R3) to the structure are defined similar to paragraph 9.

For other psychoactive substances which do not fall under the ten generic classes, but legislative controls are required due to reports of abuse or its potential threats to public health, they are listed individually in paragraph 11. Some examples of NPS which are listed in paragraph 11 are 5- and 6-APB, fluoroamphetamine, mitragynine, Salvinorin A, methoxetamine, methiopropamine, among others.

Conclusion

New psychoactive substances are emerging drugs of abuse around the world. Little is known of the pharmacology of these NPS and there is a general lack of studies on their toxicity and effects on human health. However, these NPS have been abused as recreational drugs, and many of them with dire effects. As such, there is a need to restrict the circulation of these NPS by placing them under legislative control. For forensic laboratories, there is also an increasing challenge to keep up and be informed of emerging NPS and develop methods for their analysis and identification.

References

1. *Consideration of the novel psychoactive substances ('Legal Highs')*. Advisory Council on the Misuse of Drugs (ACMD); 2011.
2. *Global Smart Update 2012*. United Nations Office on Drugs and Crime (UNODC). Vol. 8; 2012.
3. *The challenge of new psychoactive substances*. United Nations Office on Drugs and Crime (UNODC); 2013.
4. Misuse of Drugs Act (CAP. 185), Singapore. [Accessed 27 June 2013] http://statutes.agc.gov.sg
5. *Analogue controls: an imperfect law*. UK Drug Policy Commission (UKDPC); 2012.

Abbreviations

APB = 5-(2-Aminopropyl)benzofuran or 6-(2-aminopropyl)benzofuran; 1-BZP = 1-benzylpiperazine; 2C-B = 4-bromo-2,5-dimethoxyphenethylamine; 2C-I = 4-iodo-2,5-dimethoxyphenethylamine; DMMC = dimethylmethcathinone; 1,4-DBZP = 1,4-dibenzylpiperazine; FMC = fluoromethcathinone; 2-DPMP = desoxypipradrol; MA = methamphetamine; MDA = α-methyl-3,4-(methylenedioxy) phenethylamine ; MDMA = N,α-dimethyl-3,4-(methylenedioxy)phenethylamine ; MDPV = 3,4-methylenedioxypyrovalerone; MEC = methylethcathinone; 4-MMC = 4-methylmethcathinone; MPA = methiopropamine; MXE = methoxetamine; PMMA = para-methoxymethamphetamine; TFMPP = 1-(3-trifluoromethylphenyl) piperazine.

National Forensic DNA Profiling Laboratory, Bangladesh

Dr. Sharif Akhteruzzaman
National Forensic DNA Profiling Laboratory, Bangladesh

Introduction

The National Forensic DNA Profiling Laboratory is the first laboratory of its kind in Bangladesh. The laboratory was established in 2006 by the Ministry of Women and Children Affairs under the auspices of Multi-Sectoral Program on Violence Against Women. The Danish International Development Assistance (DANIDA) is also affiliated with the lab as a development partner. The main focus of the laboratory is to provide DNA profiling services to law enforcing agencies to solve violent crimes like rape or murder. As a general rule, cases involving violence against women are given preference. The laboratory, however, has gradually extended its services to solving disputes over issues related to paternity, maternity, immigration, inheritance as well as identification of missing person and disaster victim identification. Besides criminal investigative purpose, the laboratory also provides DNA services for tissue transplantation cases.

Organisation

The central laboratory is divided into four sections and supported by seven regional labs located around the country. The central laboratory is equipped with all the required facilities to carry out DNA analysis. The regional laboratories only carry out the preliminary screening of the samples and subsequently send them to the central laboratory for DNA analysis with necessary documents under a chain of custody. The four sections of the central laboratory are as follows:

- **Screening section** deals with the screening of exhibits and detects the presence of biological fluids like blood, semen, saliva by means of chemical examination, microscopic examination or by the use of ALS (Alternate Light Source).

- **DNA extraction section** isolates DNA from the exhibits that pass through screening laboratory. Various in-house and commercial methods (depending on the sample types) are used in this section for DNA isolation.

DNA Analysis Laboratory

DNA Extraction Laboratory

DNA Extraction Section

- **PCR section** is equipped with both conventional and real-time PCR. Extracted DNA is amplified by conventional PCR. Real-time PCR is used to check the quantity of DNA as well as to check for the presence of mixtures or PCR inhibitors.

- **DNA analysis section** uses various capillary electrophoresis platforms like 3100 avant or 3500 Genetic analyzer to analyse the PCR products. To evaluate a DNA profile match, software packages like GenoProof of GeneMarker HID are used.

Technologically, the laboratory is equipped with all kinds of facilities to provide DNA analysis involving autosomal STRs, Y-chromosome STRs, X-chromosome STRs or mitochondrial DNA. Since its inception in 2006, the laboratory has helped solve more than 2,000 cases from all over the country. A multitude of case types have been handled by the laboratory so far, including parentage testing, murder, sexual assault, identification, immigration dispute, sibling DNA testing and organ transplantation.

Population database

The laboratory has already established the allele frequency database for the SGM Plus, Identifiler, PowerPlex, Y-filer and ESSPlex loci corresponding to the mainstream Bengali and some other indigenous populations of Bangladesh. The Y-chromosome haplotype data has appeared in the YHRD database release 25, in 2009.

PCR Laboratory

PCR Section

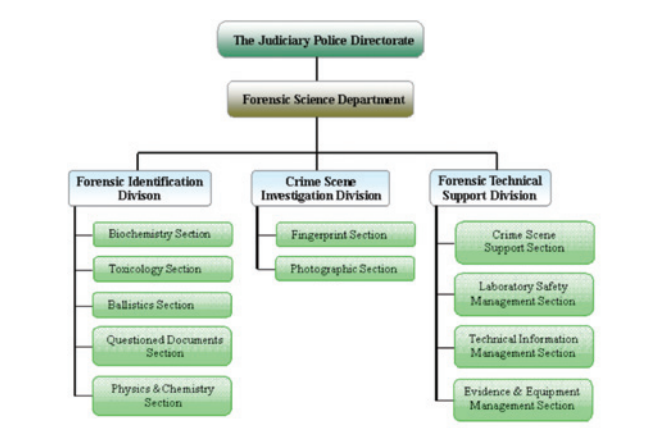
Screening Laboratory

Forensic Science Department, Judiciary Police, Macau SAR

Mr. Chio Tak Iam
Forensic Science Department, Judiciary Police, Macau SAR

Introduction

The Forensic Science Department (FSD) is the sole provider of forensic services in Macau. It is established with technical autonomy under the Judiciary Police (PJ), empowered to examine criminal evidence, conduct crime scene investigation, provide technical support and undertake research on forensic technology. Besides performing laboratory analyses on materials submitted for examination by PJ, the FSD can also collaborate with other specialised institutions, laboratories or government departments, and provide requested assistance and service to such entities on condition that the work of PJ is not being influenced.



Organisational Structure

The FSD comprises of three divisions with a team of ninety two dedicated staff members, providing a 24-hour service for efficient and precise examinations, as well as quick response to frequent urgent requests.

- **Crime Scene Investigation Division**
  The Crime Scene Investigation Division coordinates and leads crime scene investigation on a round-the-clock service. It is responsible for crime scene management, crime scene analysis, collection of scientific evidence and management of CSI equipment.

- **Forensic Support Division**
  The Forensic Support Division provides support to crime scenes which require more specialised expertise. This division is also responsible for laboratory safety, the management of submitted physical evidence, facilities and analytical instruments, the management of forensic reports and documents as well as the implementation of management with technology.

- **Forensic Examination Division**
  The Forensic Examination Division conducts forensic examinations in the field of Physics, Chemistry, Biology, of questioned documents, Illicit Drugs, Toxicology, Ballistics and the building up of forensic databases. It is also responsible for quality assurance of the examination work, carrying out research and development of new technologies. There are five sections under this division, and each of which is specialised in different forensic areas as follows:

1. *Biochemistry Section* – Utilising high-end instruments, this section conducts polymorphous DNA analysis on various biological materials such as bloodstains, semen, saliva, hair, nails and other body tissues collected in crime scenes of murder, rape, assault and hit-and-run accidents. DNA profiles obtained from the evidence can therefore be used for identification or exclusion of suspects, identification of unknown body or providing intelligent linkage for serial crimes. This section also provides paternity test service.

2. *Toxicology Section* – the primary obligation within this section is the qualitative and quantitative analyses on illicit drugs controlled by the local law. In addition, toxicological analyses for drugs of abuse and poisons in biological materials are also included in this section. Most of the cases assigned to this section are handled with urgent request.

3. *Physics and Chemistry Section* – this section is responsible for the examination of arson debris, post-blast residue, paints, corrosive chemicals, counterfeit jewellery, fibres as well as examination of marks and prints.

4. *Ballistics Section* – the key responsibilities of this section include firearms identification, range of firing estimation, gunshot residue analyses and restoration of erased serial numbers.

5. *Questioned Documents Section* – This section provides examination of handwriting and printed documents such as bank notes, credit cards, casino chips and identification documents. Forensic video enhancement is performed in this section as well.

Collaborations

With the aim to keep abreast of the forensic technologies, FSD has close contact with mainland China and Hong Kong in exchange of knowledge and experience in different forensic fields. In 2012, we are proud to become an official member of the Asian Forensic Sciences Network. We are seeking for frequent exchange of knowledge and experience as well as collaborations with experts from other member institutes.

THE UNBROKEN CHAIN – From the Crime Scene to the Courtroom

Ms. Lim Chin Chin, Ms. Chia Poh Ling, Ms. Vicky Chow Yuen San, Dr. Michael Tay Ming Kiong
Health Sciences Authority, Singapore

Introduction

The scene is typically the onset of a case investigation. In Singapore, criminal investigation is handled by the Singapore Police Force. When a distress call is received, the first responders will arrive within minutes at the scene. These police officers will secure the scene, conduct a preliminary assessment of the scene and report their findings to the Investigating Officer (IO) in-charge of the case. The scene will then be handed over to the IO and his team. Depending on the type of case and evidence encountered, the IO may activate the forensic scientist to the scene.

Crime Scene Analysis

Major scenes are usually attended by an experienced multi-disciplinary forensic scientist from the HSA Forensic Chemistry and Physics Laboratory (FCPL).

A multi-disciplinary forensic scientist is one who has broad and in-depth experience in several forensic disciplines and sub-disciplines and is knowledgeable concerning the occurrence, persistence and value of different types of physical evidence in a crime scene.

The overall success or failure of a crime scene analysis often hinges on the ability of this forensic scientist to critically assess the scene, define the problem and key issues, formulate a hypothesis, recognise the crucial pieces of evidence to collect, and identify the relevant forensic examinations that will yield information to verify or refute the hypothesis. Objective evaluation of the physical evidence present at the scene is necessary in order to gather reliable background information on the potential linkages and associations between the scene, persons and objects. First-hand, detailed examination of the crime scene allows the forensic scientist to subsequently relate, interpret and report on the different physical evidence and explain their significance in the overall context.

One must always bear in mind that anything that is not collected from the scene is lost forever. On the other hand, it is neither feasible nor practical to collect everything. Hence, a wise decision has to be made as to the:

(a) Extent of scene processing, documentation and analysis;
(b) Amount of evidence collected; and,
(c) Extent and types of laboratory examinations performed.

Submission of Evidence

Preliminary examinations at the scene by the forensic scientist may provide crucial contextual information which can shed light on an otherwise perplexing case. Nonetheless, it is not possible to perform definitive forensic analysis at the scene without the full instrumental capabilities of the laboratory. Hence, physical evidence recovered from the scene and persons involved in the crime is submitted by the IO to FCPL at HSA for detailed examinations. Sometimes, the evidence is brought back to the laboratory by the forensic scientist who attended the scene or recovered the evidence from the deceased at the HSA mortuary. For cases where the forensic scientist is not activated to the scene, the scientist will discuss the case with the IO, review his requests and provide expert advice on the value and significance of evidence which will be most useful to the case.

HSA FCPL's services are built around our core strengths in six broad and fully integrated disciplines of physical evidence:
1. Chemical analysis of unknowns and trace evidence
2. Fire and explosion evidence
3. Firearms, toolmarks, prints & impressions
4. Questioned documents
5. Bloodstain patterns, crime scene analysis and reconstruction
6. Counterfeit drugs, counterfeit medical devices and their packaging materials

Laboratory Analysis

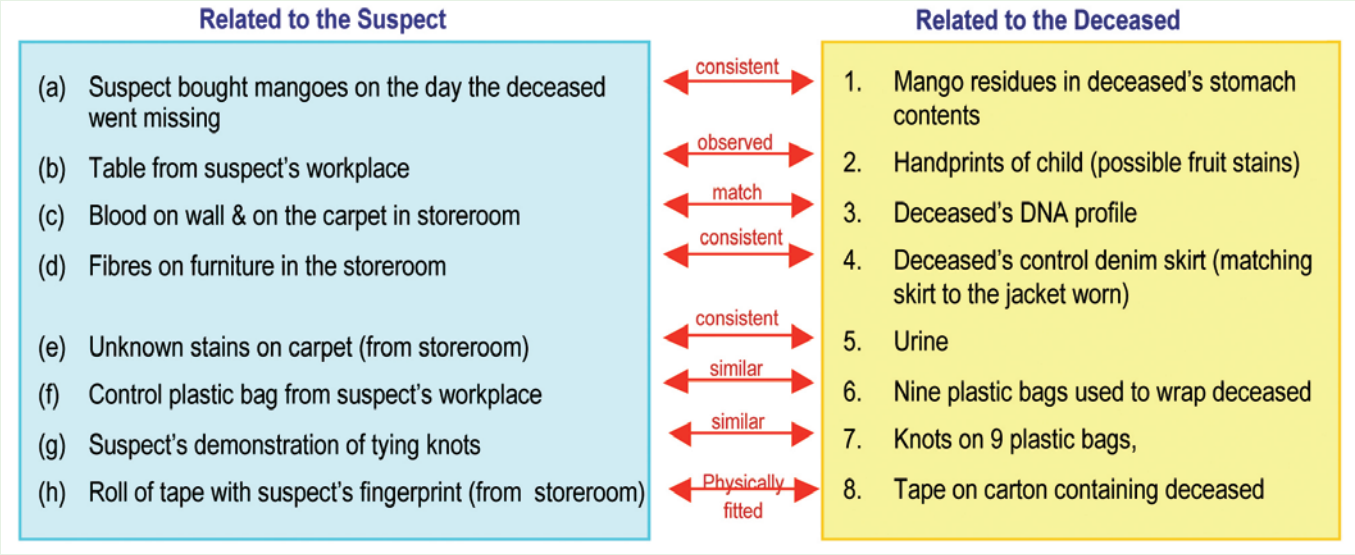
Exhibits are assigned either to a scientist or a team of scientists, depending on the types of examinations required. It is not unusual for one exhibit to have more than one type of physical evidence, thus requiring examination by forensic scientists with different expertise. A holistic approach is adopted from the start of the laboratory analysis and maintained throughout the examination process until the reporting stage. Forensic scientists discuss and decide on the sequence in which different types of evidence are recovered from the exhibits for specialised examination. Stringent protocols are adhered to regarding the chain of custody with unique identification being assigned to all exhibits and sub-samples. Sub-samples are recovered from the exhibits using various sampling techniques such as picking using forceps, tape-lifting, brushing, swabbing, solvent extraction and sieving. The recovered evidence is then analysed through a battery of macroscopic, microscopic and analytical techniques.

Trace evidence is first examined for its physical characteristics. For instance, thickness, colour, birefringence and melting point are determined for fibres; while sequence and colour of layers characterise paint fragments. Determination of chemical compositions is then performed using spectroscopic and chromatographic techniques. For the chemical analysis of unknown substances, stains and smears, the aim is to identify the unknown substance which may be present in bulk quantity or at trace level. The exhibit may be screened using physical techniques such as forensic light illumination or microscopy, and physical properties such as pH and solubility may be determined. The analyte of interest is usually extracted from the complex sample matrix for further analyses. Although a general analytical scheme is in place, the protocol is usually case-specific with several critical decision-making points that require professional judgment of the scientists. The approach varies depending on the findings and inferences obtained after each technique. Verification or validation studies are often necessary to modify a procedure for a different matrix or for a new analyte.

The examination of mark evidence pertains more to macroscopic and microscopic examinations of class and individual (random) characteristics between a possible source and the marks in question. With sufficient agreement of good class and individual characteristics, the source can be conclusively identified.

Bloodstain pattern evidence is first examined macroscopically. The physical characteristics of the exhibits and the stains are documented, followed by screening and confirmation of human blood stains. The different types of stain patterns are then identified and recorded, with appropriate stains cut or swabbed. The cuttings and swabs are then transferred to the HSA DNA Profiling Laboratory for DNA profiling.

In the case study of a 8-year old girl whose body was recovered three weeks after she was reported missing [1], the physical evidence recovered spanned several disciplines:



The yellowish-brown substance from the deceased's stomach contents was found to be consistent with mango pulp. The fluorescing prints on the table and the likely presence of mangoes in the deceased's stomach contents were consistent with the deceased eating mangoes in the storeroom. The presence of the deceased's blood on the wall and on the carpet, the presence of urine on the carpet and the significant amount of denim fibres on items found in the storeroom suggested that the storeroom was the primary scene.

The common origin of the questioned bags and the control bags found in the suspect's workplace, the similarities observed in the construction and tying order of knots, the physical fitting of the last strip of tape on the carton with the roll of tape, and the presence of the suspect's thumbprint on the roll of tape suggested that:

1. *The suspect took a stack of plastic bags from his workplace to wrap the deceased.*
2. *The suspect wrapped the body in nine layers of plastic bag, and tied similar type of knots on each bag.*
3. *The suspect used the roll of tape to seal the carton. The systematic manner in which the carton was sealed was consistent with his job as a packer.*

The meticulous and systematic manner in which the deceased was packed in the carton suggested that the suspect was in no hurry to pack the body. The findings of the stomach contents provided an estimation of the time of death.

Case study: Association between the scene, persons and objects found at the scene

trace evidence and chemical analysis (mango in deceased's stomach contents, fibres from her clothing, adhesive tape and urine stains), manufacturing marks (plastic bags used to wrap her body), prints (handprints and fingerprints), physical examinations (knots and physical fitting of tapes) and bloodstains. It was important to avoid a "silo" treatment of each type of evidence to ensure that the final report would consider the findings from each evidence type in totality [2]. The synergy between the forensic pathologist and the forensic scientist ensured that the plastic bags and knots wrapping the deceased were properly recovered and preserved. The linkages and associations between the scene, the suspect, the deceased, and objects at the scene and the suspect's workplace presented a more complete overall picture of the crime.

Reporting and Communicating Forensic Findings

Science is reliable only when the forensic scientist remains objective throughout the forensic process. The forensic scientist must be able to objectively interpret the results obtained from the analysis of multiple evidence types, identify the associations between the exhibits, and explain the significance of these findings clearly in his report with appropriate use of qualifiers and statement of any assumptions used.

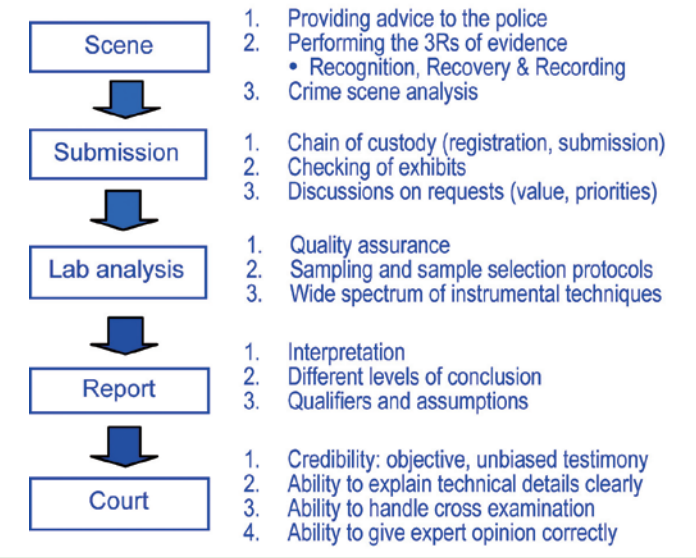
The conclusion comprises statements that the evidence supports. The level of conclusion increases with the number of similar characteristics within an evidence type, for example, when different types of matching fibres are found, or when significant individual characteristics are found on both the known shoe and the questioned shoeprint. The level of conclusion further increases with associations of different evidence types; for example, when definitive findings for paint evidence, glass evidence and damage analysis are correlated and consistent in a hit-and-run case report.

Identifying a material answers the question "What is it?". Comparative analysis further answers the question "Where did it originate from?" E.g. identifying tiny fragments as possible vehicular glass fragments. Further comparison with known vehicles to determine whether they could have originated from the broken windscreens. The activity level sheds light on how the glass fragments could have landed on the suspect's clothing. i.e., the suspect was near the vehicles when the windscreens were smashed.

Two types of conclusions are reported: case reports that report at the source level and those that look at the activity level [3]. The source level identifies the chemical composition and origin of the material. The activity level provides information on the event which likely occurred, resulting in the presence of the material.

For complex cases, forensic scientists responsible for the different types of evidence will each issue a report based on their respective areas of expertise and one senior multi-disciplinary forensic scientist will generally collate all the findings from these reports and correlate them in a single report. Sometimes, this integrated scientific interpretation of evidence draws on additional findings from crime scene analysis, DNA profiling, medical, autopsy and toxicology reports, giving rise to a Crime Scene Reconstruction report. This is an overarching report that integrates all the information from the various expert reports to establish the likely events which could have occurred at the scene(s).

When the IO collects the completed case reports, it is useful for the forensic scientist to conduct a case discussion on the significance of the laboratory findings, so as to assist the IO in



A flowchart showing the unbroken chain - from the scene to the courtroom.

understanding the scientific findings, the value of the evidence and how it can be used in Court to support or refute statements made by persons associated with the case.

Presenting Court Testimonies

This is the final stage of the entire chain, one whereby the forensic scientist is often put under the scrutiny of the Courts, the public and the media. The ability of a forensic scientist to articulate highly technical findings in a clear, concise and logical manner and to provide expert opinions based on his findings and experience will be essential to complete the scientific process. This ability will be greatly enhanced if prosecutors have a clear understanding of the scientific findings before the trial and if the forensic scientist could use appropriate tools to explain his findings in court.

In Singapore, the prosecutor usually conducts interview sessions with the forensic scientist before the court trial to better understand the scientific report findings and their significance. PowerPoint presentations are often used in the courtroom by forensic scientists for complex cases to better explain the scientific findings and occasionally, a mock-up scene using custom-made furniture is set up in the courtroom to better illustrate a postulation or an event/scenario.

At the very end, one must always remember that the role of a forensic scientist is not to specifically support prosecution or defence. Our scientific contribution to the administration of justice is to produce reliable, reproducible and robust scientific findings and to objectively provide the court with an expert opinion of the meaning of the evidence. As Dr P.C.H. Brouardel wisely put it: “If the law has made you a witness, remain as a man of science. You have no victim to avenge, no guilty or innocent person to convict or save. You must bear testimony within the limits of science.”

Conclusion

In the past decade, FCPL has successfully implemented a holistic, interdisciplinary and team-based approach in evaluating and integrating the diverse types of physical evidence encountered in a case. This approach has been championed by pioneer criminalists such as James Robertson [2], Peter de Forest [4, 5] and Jerry Chisum [6], who have for years advocated that the forensic scientist must think critically beyond narrow confines, ask relevant scientific questions to clarify and define the problems to be solved rather than examine only what is requested in the submission forms, evaluate the physical evidence aspects of a case in the totality of the event, and interpret holistically the various types of evidence, especially in complex reconstruction cases. Similar views were expressed by veteran FSS forensic scientists Stephen Day [7], Jeffrey Gray and Sara Gray [8] in the UK Parliament. Likewise, T.A. Tsoi of the Netherlands Forensic Institute has cautioned against fragmentation of forensic expertise into numerous specialised areas that rarely engage in deep interdisciplinary cooperation [9].

Experience of FCPL demonstrates that the holistic and synergistic approach that integrates all evidence associated with a case is not only feasible but also of great benefit to the outcome of the forensic process. This unbroken chain vastly enhances overall evidential value due to the potent multiplier effect achieved by combining the value of the diverse types of physical evidence.

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A Method for Acquiring IMSI from Android Phones

Mrs. Kang Yan Rong[1], Mr. Fan Wei[2], Mr. Zhou Dong Lin[1], Mrs. Guo Li Li[1], Mr. Li Xiao Lu[1]
[1] Institute of Forensic Science, Ministry of Public Security, China
[2] Institute of Forensic Science, Tianjin Public Security Bureau, China

Abstract

Using IMSI to track down suspects is a form of case investigation. In this paper, we present a method for acquiring IMSI from Android phones. The method uses forensic tools to obtain authority and extract file system data from Android phones, following which the IMSI can be found in the xml file in the shared preferences folder (com.android.phone_shared_preferences.xml).

Introduction

The International Mobile Subscriber Identity (IMSI) number provides a unique identification of the customer. It is also used to connect mobile phone calls between the SIM card and the network, and identifies the individual operator network. Using Sim Manager Pro (previously Sim-Surf Profi), ChipIt, PDUSpy and SIM-Scan, Cards4Labs [1], the IMSI can be acquired from the SIM card. Curran *et al*. proposed a method that unlocks and re-programmes boxes to obtain a binary file of the SIM card, thus allowing the IMSI to be found by analysing the binary file [2]. Reiber introduced a method to use the existing ICCID to analyse the IMSI [3]. In this paper, we propose a new method of acquiring IMSI from mobile phones.

Materials and Methods

Phone samples

This study selected four types of Android phones, as shown in Table 1:

Number	Phone Model	System Version	Remarks
1	Samsung I9300	Android 4.0.4	
2	Samsung 5830I	Android 2.3.6	
3	HTC ONE	Android 4.0.4	
4	HTC S710D	Android 2.3.4	Telecom customised

Table 1: Basic information of Android phone samples.

Methods

At present, the three methods used to extract the mobile phone data are via logical extraction, file system extraction and Bit-to-Bit extraction. Logical extraction allows an investigator to extract the logical files of mobile devices as a directory structure that does not include unallocated space and deleted files. File system extraction allows an investigator to gain access and recover hidden databases which cannot be accessed by other file system acquisition tools. Bit-to-Bit extraction allows an investigator to create a physical image of the mobile device's flash memory or address range, which also includes unallocated space where you will be able to find deleted content [4]. This experiment used a Universal Forensic Extraction Device (UFED) v1.8.6.69 to connect with four mobile phones, acquire the operating authority and extract data by file system dump. IMSI can be acquired from IMSI_Item as shown in Table 2.

No.	Phone Model	IMSI_Path	IMSI_ Item
1	Samsung I9300	\Dump\data\data \com.android.phone \shared_prefs \com.android .phone_shared_preferences. xml	cf_imsikey
2	Samsung 5830I	\Dump\data\data \com.android.phone \shared_prefs \com.android .phone_shared_preferences. xml	cf_imsikey
3	HTC ONE	\Dump\data\data \com.android.phone \shared_prefs \com.android .phone_shared_preferences. xml	sim_imsi_key
4	HTC S710D	\Dump\data\data \com.android.phone \shared_prefs \com.android .phone_shared_preferences. xml	sim_imsi_key

Table 2: IMSI storage location of Android phones.

Results and Discussion

In this experiment, the four mobile phones were initially in their factory setting status. The com.android.phone_shared_preferences.xml of Samsung mobile phones had been formed in the factory setting status, but it was only formed in HTC mobile phones after inserting the SIM card. The experiment also found that the com.android.phone_shared_preferences.xml location only stores IMSI of the last inserted SIM card.

Comparing the method in this paper to Cellebrite logical extraction and XRY software, if the mobile phone is not rooted, these software are only able to extract the last IMSI, so we can use the method mentioned in this paper to extract all the IMSI.

Conclusion

In this paper, we have presented a method of IMSI acquisition from system files that can be obtained by file system extraction. For future work, we will research how to acquire IMSI from the image files that can be obtained by Bit-to-Bit extraction.

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Assessment of Stochastic Variations in Low Template STR Profiles

Dr. Seah Lay Hong, Ms. Dharmeziyana Daud, Mr. Abdul Hail Bin Ahmad Tarmizzi
Department of Chemistry, Malaysia

Abstract

The increasing sensitivity of STR typing kits to detect low levels of DNA has led to an increasing number of partial DNA profiles and accompanying stochastic variation characterised by heterozygote imbalance and allelic drop-out. The existence of allelic drop-out becomes probable when a single allele is observed at below the stochastic threshold. Using the AmpFlSTR® Identifiler® Plus amplification kit, the STR profiles (carried out in duplicates and by two analysts) for DNA templates ranging between 500 pg to 1.9 pg (from an individual heterozygous at all AmpFlSTR® Identifiler® loci) were examined. Based on the findings of this study, a number of considerations have been proposed for STR typing with low template DNA.

Introduction

When there is sufficient DNA to generate peaks above the stochastic threshold, the two alleles of a heterozygote pair are balanced and a match between the donor and the crime stain can be easily read. As the template DNA level decreases, the signal level decreases and the heterozygote balance deteriorates because of stochastic or random effects [1,2]. Allele drop-out is an extreme example of heterozygote imbalance, where one allele falls below the limit of detection threshold. Many laboratories, including the DNA laboratory in the Department of Chemistry, Malaysia, have typically set this detection limit to 50 rfu (relative fluorescent unit). The inevitable consequence of allelic drop-out is that the crime-stain DNA profile may not match the DNA profile of the hypothesised contributor.

The stochastic threshold rules that for a single allele below the stochastic threshold there is uncertainty about the genotype for the particular locus. There is probability that an unseen companion allele has dropped out [3, 4]. The critical issue is that for homozygous peaks below the stochastic threshold, there is uncertainty about whether this is a true homozygous peak.

Low levels of DNA may also cause the detection of additional alleles or drop-ins. The drop-in phenomenon was originally described by Gill *et al*. [5] and unlike contamination, is reportedly restricted to 1 or 2 alleles per profile.

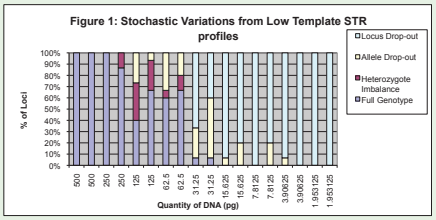
Materials and Methods

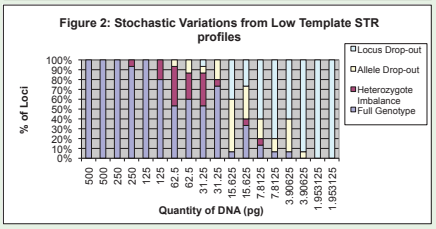
A 1000 pg quantity of DNA (nominal estimate only) from an individual who is heterozygous at all AmpFlSTR® Identifiler® Plus loci was serially diluted down to 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90625 and 1.953125 pg. These nine different amounts of DNA were amplified in duplicate using the AmpFlSTR® Identifiler® Plus amplification kit for 28 PCR cycles. The amplicons were separated on an Applied Biosystems 3130xl Genetic Analyzer with CE injection at 3 kV/10 s and analysed using GeneMapper ID v3.2.1 software. A detection threshold limit of 50 rfu was used to designate alleles. The experiments were carried out in duplicate by two analysts, thus four sets of STR results were produced.

Results and Discussion

The STR results for DNA templates ranging between 500 pg to 1.9 pg (from an individual heterozygous at all AmpFlSTR® Identifiler® loci) were summarised in Figures 1 (Analyst 1 in duplicate) and 2 (Analyst 2 in duplicate). Heterozygous alleles with ratios less than the standard 0.6 proportion guideline were indicated as heterozygote imbalance.

Heterozygote imbalance with heterozygote ratios of < 0.6 were observed in samples with DNA templates of 250 pg and less of DNA. Allelic drop-out was first detectable at 125 pg DNA templates and locus drop-out appeared at 31.25 pg and less of DNA templates. Genotyping





completely failed with 1.95 pg and less of DNA templates. No drop-ins were observed in any of the low-template STR profiles. Among the allelic drop-outs observed, the maximum observed peak height for the surviving allele is 148 rfu at locus D18S51.

For determination of the stochastic threshold, a larger number of low template DNA samples are required. For practical use stochastic threshold is chosen at a relative fluorescent unit value for which 99% of the single alleles on heterozygous loci were below it [6].

For purposes of casework with low template DNA, the following guidelines are recommended:

1) Because of stochastic variation, any apparent homozygote is considered to be a potential heterozygote.
2) The increased heterozygote imbalance means that the 0.6 proportion guideline used in the interpretation of standard STR profiles cannot be used for identifying mixtures in low template DNA profiles.
3) The use of 28 PCR cycles for AmpFlSTR® Identifiler® Plus amplification with low template DNA is expected to be a reliable cycle number for diminishing the risk of drop-ins.
4) The 50 rfu threshold for allele calling of low template DNA profiles remains a reliable threshold for designating alleles.
5) A stochastic threshold may not be used for low template DNA profiles as there is always the need to consider the probable occurrence of drop-outs. Multiple PCR amplifications are indicated if there is one allele in a sample which does not match the DNA profile of the hypothesised contributor.

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Identification of Human War Remains by Next Generation Sequencing Method on Mitochondrial DNA

Dr. Nguyen Van Ha
Institute of Forensic Science, Ministry of Public Security, Vietnam

Abstract

The long and devastating Vietnam War resulted in the loss of many lives. There are approximately 320,000 human remains resulting from this war still yet to be identified; this is of great concern to many of the Vietnamese families who lost their family members in the war. Currently, Short Tandem Repeat (STR) technology is used in war remains identification; however, due to the limitation of STR technology in its application on degraded DNA, the success rate is low. This study utilises mitochondrial DNA (mtDNA) which is abundant in highly degraded samples and its ability to trace maternal lineage making it ideal for human identification. This study demonstrates the feasibility of using Illumina's next generation sequencing (NGS) technology to sequence the 3.3 kb mitochondrial D-loop regions in the identification of human remains.

Introduction

Three long and devastating wars with France, the United States and China have left about 320,000 remains buried in 3,000 cemeteries in Vietnam. However, the authenticity of the remains has always been a question to their families. Besides that, there are about another 226,000 remains whose identities cannot be determined. In total, there are 546,000 remains which need to be identified including soldiers from the United States, Korea, Thailand, Philippines, and Australia who died in the war. In June 2013, the remains of a U.S. Air Force pilot listed as missing in action since his plane crashed in Laos in 1967 had been identified using circumstantial evidence, dental comparisons and mtDNA testing. His remains were returned to his family for burial with full military honors.

As a Buddhist country, families with missing family members from the war were eager to identify their relatives' remains and bury them in their family graves as a form of respect. Currently, many of the families search through their relative's remains by visual identification and DNA analysis by STR genotyping. Both are equally challenging with low success rates due to the huge number of unidentified war remains and highly degraded DNA. Therefore, a fast and sensitive method of analysing degraded DNA is a greatly sought for. We have therefore chosen to explore the approach of mtDNA NGS sequencing using the Illumina MiSeq system to overcome the challenges.

Mitochondrial DNA is an ideal source of genetic material when nuclear DNA is not available or is too degraded for analysis. It is almost exclusively inherited from the mother and therefore is also a useful resource in human identification. The mitochondrial genome is about 16kb long and contains two main regions: a control region/D-loop (non coding region) and a coding region. There are two hypervariable segments in the control region, HV1 and HV2, which are informative in the identification of individuals (Figure 1).

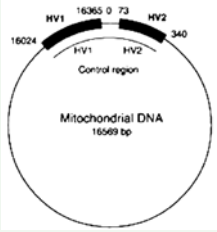

Figure 1: Diagram of Mitochondrial DNA with HV1 and HV2 regions.

Materials and Methods

DNA was extracted from bones and teeth collected from the human remains of those who perished during the Vietnam War. Blood samples were collected from their relatives as a reference. The HV1 and HV2 regions of mtDNA were amplified using 8 PCR primers resulting in 4 contigs (PSI, PSII, PSIII, PSIV). The amplicons were processed using Nextera XT kit according to the manufacturer's protocol. Samples were sequenced at 2x 151bp on a Illumina MiSeq system. The raw sequences were aligned to mtDNA reference sequence using the BWA algorithm and variant-calling was generated using GATK. The samples were compared based on single nucleotide change profile.

Results and Discussion

The relationships of samples were evaluated based on single nucleotide change profiles. The DNA standard 9947A was used as a positive control in the study. The variants called in 9947A were compared to the known SNPs in the database. The results showed that all the known calls were able to be identified with near 100% frequency (Table 1 and Figure 2).

POS	Known call type	Frequency
93	A->G	99.8%
195	T->C	100.0%
214	A->G	99.8%
263	A->G	99.8%
750	A->G	100.0%
1438	A->G	99.9%
15326	A->G	99.6%
16311	T->C	100.0%
16519	T->C	99.9%

Table 1: Variant calls of 9947A were compared to known calls in the database. The results showed close to 100% frequency in calling.

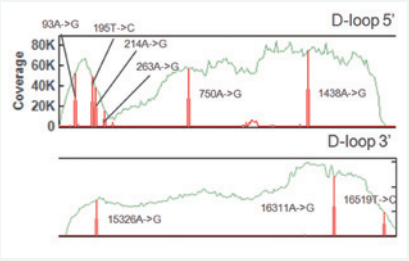

Figure 2: Single nucleotide change profile of 9947A.

Sample 995A and 995B are war remains and reference DNA, respectively. The results of the single nucleotide change profile from 995A (teeth from remains) and 995B (blood sample from 995A's maternal relative) showed perfect concordance indicating these samples have the perfect relationship. An un-related sample showed a distinct profile (Figure 3).

Figure 3: Single nucleotide change profiles of 995A (teeth from remains) and 995B (blood sample from 995A's maternal relative) and an un-related sample at the D-loop region. Samples 995A and 995B have identical profiles while the un-related sample has a distinct profile.

In our second attempt of sequencing, samples Me (blood), Huyen (blood), and Thao (blood) are maternally related, while samples 1559B (blood), 1559C (blood) and Xuong (bone) are un-related. The results clearly showed that Me, Huyen and Thao have identical profiles at both the HV1 and HV2 loci while the un-related samples have distinct profiles (Figure 4 and 5). One notable result from Xuong in HV2 was low variant calling frequency which could be due to poor DNA quality extracted from the bone sample.

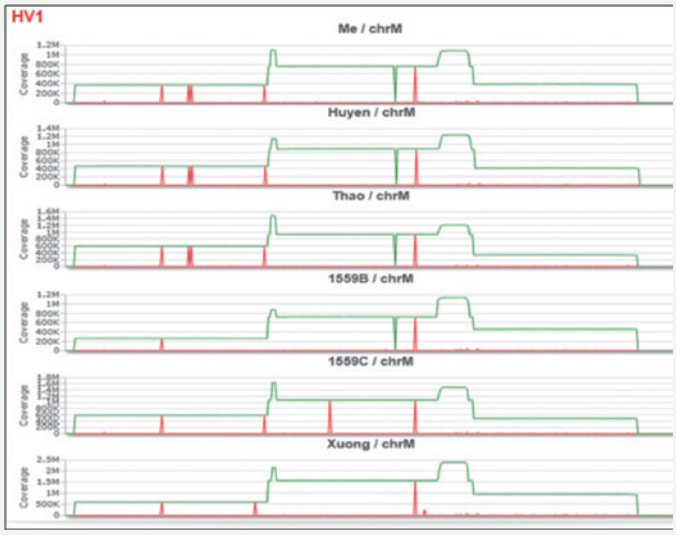

Figure 4: Single nucleotide change profiles from Me (blood), Huyen (blood), Thao (blood), 1559B (blood), 1559C (blood) and Xuong (bone) at the HV1 region. Me, Huyen and Thao have identical profiles while the remaining 3 have distinct profiles.

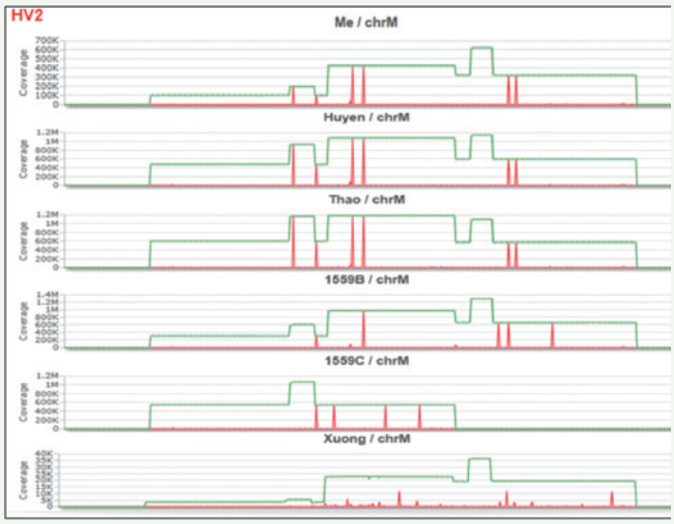

Figure 5: Single nucleotide change profiles from Me (blood), Huyen (blood), Thao (blood), 1559B (blood), 1559C (blood) and Xuong (bone) at the HV2 region.

Conclusion

We demonstrated the feasibility of using MiSeq and mtDNA sequencing in human identification. Control 9947A showed close to 100% frequency in SNP detection concordance to a public database. All variants identified from bone extract 995A were concordant with the matched blood sample (995B) confirming the relationship between the 2 samples. The maternally related samples were from non-related samples based on the single nucleotide change profiles which were identical in the former but not the latter. We also found that while bone samples work reasonably well in mtDNA analysis, the mtDNA obtained from bone may still be degraded resulting in a reduction of the desired signal and increased observation of noise. We hope to further optimise the extraction protocol to improve yield and sensitivity of sequencing while reducing costs by increasing the multiplexing in the NGS.

Sibship Analysis by AmpFlSTR® Identifiler® Amplification System in the Chinese Han Population

Ms. Guo Yanxia, Ms. Kang Yanrong, Mr. Chen Song, Mr. Li Wanshu, Mr. Liu Kaihui, Mr. Zhou Yi, Ms. Hao Jinping, Dr. Ye Jian
Institute of Forensic Science, Ministry of Public Security, China

Abstract

Using the AmpFlSTR® Identifiler® Amplification system, sibship analysis was carried out on 151 pairs of full siblings from the Chinese Han population. The probability of full siblings (W_{FS}) and the number of matching alleles for each locus was compared between full siblings and unrelated individuals in the Chinese Han population. This study demonstrated the utility of the AmpFlSTR® Identifiler® System in differentiating between full siblings and unrelated individuals.

Introduction

DNA profiling of individuals with short tandem repeats (STR) is commonly used in forensic genetics and paternity testing. This technique can be used as well for forensics and immigration purposes in the establishment and settlement of family relation problems [1]. AmpFlSTR® Identifiler® Amplification System is also commonly used in the Asian forensic laboratory.

This research focuses on the analysis of full siblings for the purpose of setting a statistical threshold to establish if two individuals are true full siblings. To meet the demands to solve some complex full siblings identification cases in which parentage references are lacking and beyond parent-child paternity identification [2], we investigated the use of the AmpFlSTR® Identifiler® typing system as a tool to differentiate between full siblings and unrelated individuals. The Automatic Analysis Software of Sibling Identification (ASI) software, an in-house patented software, was utilised as a statistical tool [3].

Materials and methods

Samples

DNA specimens from 151 pairs of full siblings and 31,224 simulated pairs of unrelated individuals were typed using the AmpFlSTR® Identifiler® Amplification kit.

STR-analysis

All samples were analysed at all 15 autosomal STR loci in the AmpFlSTR® Identifiler® Amplification kit. Samples were amplified in an ABI 9700 thermal cycler and separated with an ABI Prism 3130XL Genetic Analyzer according to the manufacturer's instructions. Data was analysed with GeneMapper® ID 3.2 and could be input-output automatically or manually from ABI Prism 3130XL Genetic Analyzer to ASI software by the programme we designed. Different threshold values can be implemented into the software according to each criminal case to find clues on suspects, victims etc.

Statistical Analysis

Sibship index of full siblings (PI_{FS}) and sibship probability of full siblings (W_{FS}) were calculated with the ITO method as commonly used in forensic sibship identification. The relationship is $W_{FS} = PI_{FS}/(PI_{FS}+1)$. W_{FS} is used and accepted more commonly than PI_{FS} in our forensic identification reports. Allele frequencies were calculated by simple counting and tests for Hardy-Weinberg equilibrium and linkage disequilibrium were carried out using our patented ASI software presented [4].

Results

The ASI software has been programmed to calculate paternity probability (W_{FS}) and the number of matching alleles based on the Chinese Han population.

W_{FS} (%)	full sibling			unrelated individuals		
	case	Accumulated case	Accumulated percentage	case	Accumulated case	Accumulated percentage
≥ 99.99999	19	19	12.58%	-	-	-
> 99.9999	15	34	22.52%	-	-	-
> 99.999	25	59	39.07%	-	-	-
> 99.99	27	86	56.95%	1	1	0.00%
> 99.9	29	115	76.16%	2	3	0.00%
> 99	15	130	86.09%	25	28	0.09%
> 90	16	146	96.69%	168	196	0.63%
> 10	4	150	99.33%	2200	2396	7.67%
> 1	1	151	100.00%	4242	6638	21.3%
≤ 1	-	-	-	24586	31224	100%

Table 1: Distributions of W_{FS} in full sibling individuals and unrelated individuals under the different critical boundary

In total 151 pairs of full siblings and 31,224 pairs of unrelated individuals from the Chinese Han population were analysed [5, 6]. As shown in Table 1, sibship probability of full siblings (W_{FS}) of 39.07% full siblings were more than 99.999%, while none of the unrelated individuals computed a W_{FS} of more than 99.999%. W_{FS} of 60.93% full siblings were at the range from 99.999% to 1%, while W_{FS} of 21.3% unrelated individuals were at the range from 99.999% to 1%. W_{FS} of 78.7% unrelated individuals were less than 1%, while none of the full siblings pairs had W_{FS} less than 1%. Thus, there was a notable difference in the W_{FS} between full siblings and unrelated individuals.

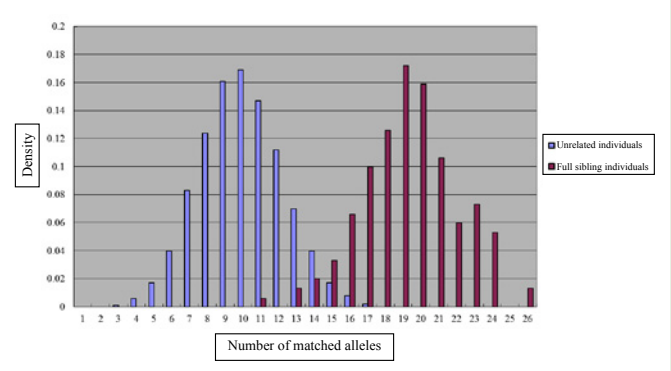

Figure 1: Distributions of the matched allele in full sibling individuals and unrelated individuals

From Figure 1, we also found that the number of matching alleles were evidently greater among the full siblings pairs than among the unrelated individuals. The matched allelic numbers of unrelated individuals was about 3-17 in AmpFlSTR® Identifiler® System, however, the numbers to full sibling individuals was about 11-26. Among the range of 11-17, full sibling individuals and unrelated individuals have the same distributions of the matched alleles. The distributions of the number of matching alleles in full sibling individuals and unrelated individuals approximates to a normal distribution.

The number of matching alleles for all loci is also elucidated in Table 2 and Table 3 which further indicated a notable difference in distributions between full siblings pairs and unrelated individuals.

allelic numbers	full sibling individuals (n=151)			unrelated individuals (n=31224)		
	entirely-same	half–same	entirely-different	entirely-same	half–same	entirely-different
0			32	8288	3	13
1	1		47	11735	6	104
2	3	1	44	7457	110	448
3	11	1	18	2827	445	1440
4	23	7	7	745	1352	3274
5	32	10	2	147	2944	5246
6	32	23	1	24	4888	6568
7	17	28		1	6178	5941
8	15	29			6092	4489
9	12	31			4723	2371
10	2	13			2780	951
11	3	6			1186	306
12		2			384	64
13					112	8
14					21	1

Table 2: Allelic matching results in full sibling individuals and unrelated individuals. X^2 = 33370.72, P<0.001 entirely-same; X^2 = 31026.57, P<0.001 (entirely-different).

forecast result	assumed boundary		Wrong forecast cases	Wrong forecast rates (%)
unrelated individuals	entirely-same	=0	--	--
		≤1	1	0.66
		≤2	4	2.65
	entirely-different	≥7	--	--
		≥6	1	0.66
		≥5	3	1.99
full sibling individuals	entirely-same	≥8	--	--
		≥7	1	0.00
		≥6	25	0.08
		≥5	172	0.55
		≥4	917	2.94
	entirely-different	=0	13	0.04
		≤1	117	0.37
		≤2	565	1.81

Table 3: Probability of wrong forecast in different critical boundary

Discussion

This study suggests that W_{FS} and the number of matched alleles may play an important role in sibship analysis for full siblings based on Chinese Han population by Identifiler system [7].

We found that when the W_{FS} of two individuals is more than 99.999%, they can be considered as being full siblings. If the W_{FS} of two individuals is between 99.999% to 1%, more STR markers are required to enhance accuracy of the test result, such as other kits like Y-STR, X-STR and mitochondrial DNA etc. If the W_{FS} of two individuals is less than 1%, they can be excluded as being full siblings [8].

We also found that for a pair of individuals to be considered full siblings, it is required that both alleles of each locus are entirely the same for at least five loci or that both alleles of each locus are entirely different for not more than 1 locus. A pair of individuals could be considered unrelated when both alleles of each locus are entirely different for at least 6 loci or both alleles of each locus are entirely the same for not more than 1 locus.

From the findings of this study, we outlined the recommendations described above to be adopted as a guideline for differentiation between full siblings and unrelated individuals based on the AmpFlSTR® Identifiler® typing system and the ASI software [9]. The ASI software may also be used to build a database with Chinese Han frequencies to join national familial searching software [10], which is an investigative technique useful when a database search does not yield a full match to a crime scene profile.

Acknowledgement

This work was supported by the National Basic Research Fund of Institutes.

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The Analysis of a Case with Mixture Sample Using the Restricted Combinatorial Model Method

Ms. Zhao Lei, Mr. Sun Jing, Ms. Ou Yuan, Prof. Zhao Xingchun, Ms. Li Caixia, Prof. Ye Jian
Institute of Forensic Science, Ministry of Public Security, China

Introduction

A DNA mixture profile obtained from casework was analysed using the restricted combinatorial model method for mixture interpretation, described in the recommendation of the International Society of Forensic Genetics in 2005. It entails a series of steps including identifying the number of contributors, estimating the mixture proportion of individuals in relation to the mixture, evaluating the heterozygote balance, considering all possible genotype combinations and lastly comparing reference sample before arriving at a probable genotype combination of individuals in the mixture.

Materials and Methods

The sample was from one of the routine rape cases. DNA was extracted by Chelex, amplified and the PCR products were separated by capillary electrophoresis on ABI 3130 Genetic Analyzer. The results were analysed by GeneMapper® Software Version 3.2.

Results

Inference on the number of contributors in the mixture

The DNA profile in Figure 1 showed that it was likely to be a two-person mixture as there were a maximum of four allelic bands at each locus. Using the gender locus peak area as a rough estimate, a major female contributor and a minor male contributor could be observed.

Analysis of heterozygote balance (H_b) and mixture proportion (M_x) using gender locus peak area

The H_b minimum threshold in this case study was set at 0.6 and the maximum at 1 (i.e. $0.6<H_b<1$) and the peak areas of allele X from the male contributor were estimated using the peak area of allele Y (ϕ_Y=3074rfu). The formulae were: $\phi_X^{Y}{}_{min1} = \phi_Y \times H_{b\,min} = \phi_Y \times 0.6$ and $\phi_X^{Y}{}_{max1} = \phi_Y \times H_{b\,max} = \phi_Y \times 1$ (assuming allele Y was the major allele in the male contributor) and $\phi_X^{Y}{}_{min2} = \phi_Y / H_{b\,max} = \phi_Y / 1$ and $\phi_X^{Y}{}_{max2} = \phi_Y / H_{b\,min} = \phi_Y / 0.6$ (assuming allele X was the major allele in the male contributor). The range of ϕ_X^{Y} worked out to be 1844.4 rfu – 3074 rfu and 3074 rfu – 5123.3 rfu respectively.

In this case study, the estimated minimum and maximum M_x for each assumption were calculated. The formulae were: $\hat{M}_X^{Y}{}_{min1} = \frac{f_Y + f_X^{Y}{}_{min1}}{f_X + f_Y}$ and $\hat{M}_X^{Y}{}_{max1} = \frac{f_Y + f_X^{Y}{}_{max1}}{f_X + f_Y}$ when assuming allele Y was the major allele in the male contributor

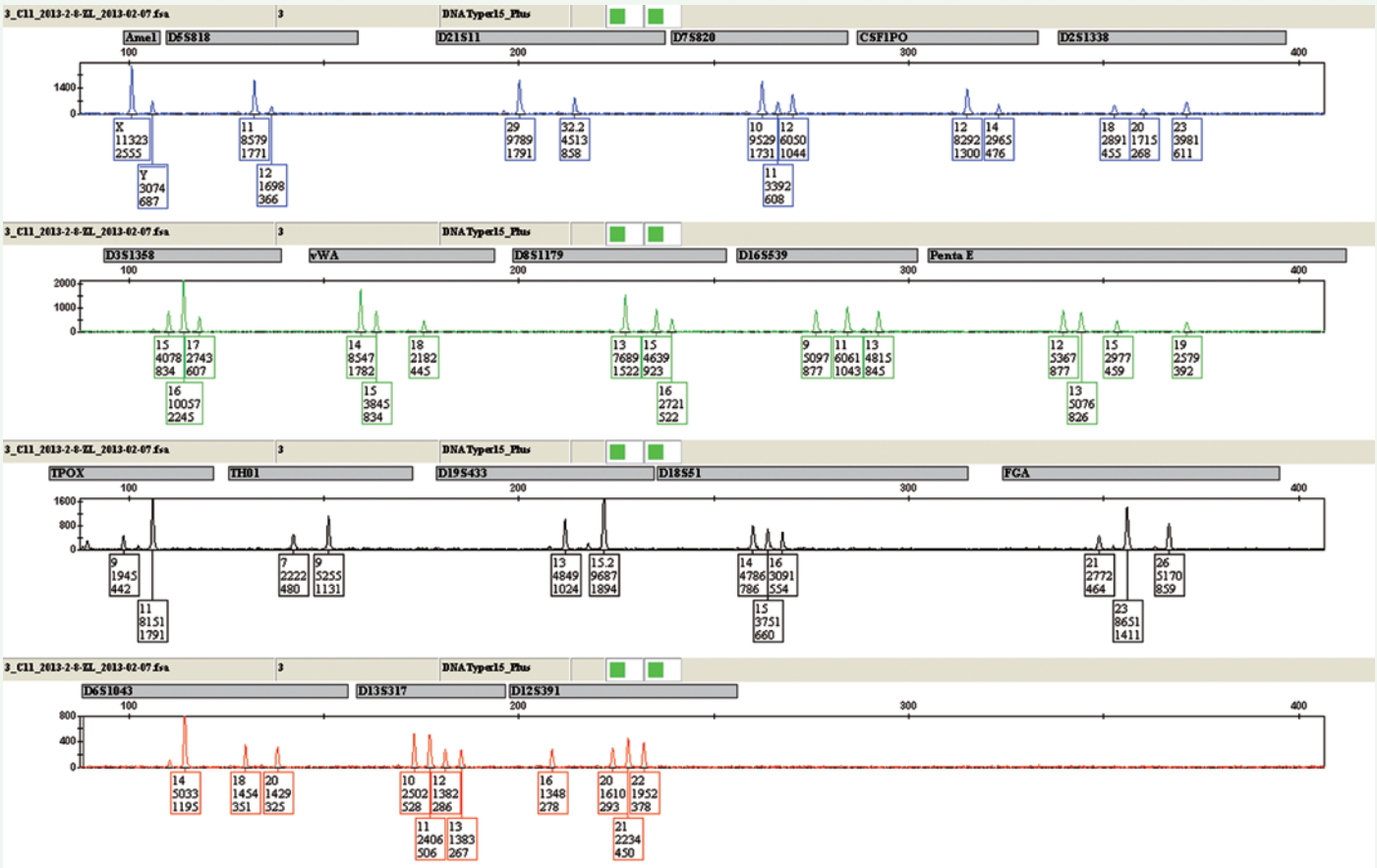

Figure 1: The DNA profile of the mixture from the case.

and $\hat{M}_X{}^{Y}{}_{\min 2} = \frac{f_Y + f_X{}^{Y}{}_{\min 2}}{f_X + f_Y}$ and $\hat{M}_X{}^{Y}{}_{\max 2} = \frac{f_Y + f_X{}^{Y}{}_{\max 2}}{f_X + f_Y}$ when assuming allele Y was the minor allele [1, 2]. The $\hat{M}_X$ of the gender locus in this mixture ranges from 34%-42% and 42%-57%, which means the $\hat{M}_X$ of the male contributor in the mixture is 34% to 57%.

The analysis of all possible genotype combinations

The heterozygote balance between any two alleles and the mixture proportion for each genotype combination across all loci was calculated. The probable genotype combination was selected according to both conditions: $0.6<H_b<1$ and $\hat{M}_X$ between 34% - 57%. The results for D5S818, D7S820 and PentaE were listed in Table 1 as examples.

The comparison with reference sample

Generally, the possible genotypes could be more than one combination based on the calculation of heterozygote balance and mixture ratio. Therefore, it is important to compare the results with the victim's profile in order to ensure a well-supported analysis of the suspect's DNA profile.

Loci	Allele	Peak area	H_b	The possible genotype combinations with H_b>0.6	M_x	The probable genotype	
						Female	Male
D5S818	11	8579	$Hb_{11,12}$= 0.20	11,12 or 11	26% - 44%	11	11,12
	12	1698					
D7S820	10	9529	$Hb_{10,11}$= 0.35	10,12 or 10,11	29% - 48%	10,12	10,11
	11	3392	$Hb_{10,12}$= 0.63	10,12 or 11	18%		
	12	6050	$Hb_{11,12}$= 0.56				
PentaE	12	5367	$Hb_{12,13}$= 0.94	12,13 or 15,19	35%	12,13	15,19
	13	5076	$Hb_{12,15}$= 0.55				
	15	2977	$Hb_{12,19}$= 0.48				
	19	2579	$Hb_{13,15}$= 0.58				

Table 1: Assessment of genotype combinations relative to M_x and H_b.

Discussion

The peak area of each allele in this paper was studied for the analysis of the possible genotypes [3]. Heterozygote balance (H_b) and the mixture proportion (M_x) were employed in the calculation of mixture. H_b refers to the fluorescence intensity ratio between the shorter allele and the taller allele and was shown to be greater than 0.6 when the DNA template was not degraded and more than 500 pg [1, 4]. The gender locus contained more information about the individual and the typing result was easy to analyse, so it was selected as the standard. When H_b between the two alleles is greater than 0.6, it means they are likely to come from the same individual; when it is less than 0.6, it means they are likely to come from different individuals. M_x means the ratio of one donor's DNA in relation to the mixture DNA. It is an estimated value and can be calculated using allele peak area. In this paper, the genotypes were analysed using H_b and M_x based on the restricted combinatorial model method. The probable genotype combination was selected by evaluating the ratio of male DNA.

The method is relatively scientific, but forensic scientists also need to consider the impact of stutters, allelic drop-outs and low copy numbers. Meanwhile, the method should be extensively applied in the calculation of DNA mixture. We would use this method on more complex mixtures in future research.

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Herbal Products and Ecstasy Tablets Containing Legal Highs Encountered in Malaysia

Dr. Vanitha Kunalan, Ms. Nurhazlina Hamzah
Department of Chemistry, Malaysia

Introduction

Legal highs are designer drugs that are produced by synthetic methods and are used as recreational drugs. These drugs are synthesized from precursor substances that are readily available. Like amphetamine type stimulant (ATS) drugs, legal highs are psychoactive stimulants but at the same time, being able to circumvent existing legislation, they are legal to possess in certain countries. It appears in various forms such as tablets, capsules and herbal products. Recent examples of legal highs include synthetic cannabinoids and synthetic cathinones. In this study, we report the analysis of several synthetic cannabinoids in herbal products and synthetic cannabinoids with synthetic cathinones in tablets and capsules using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for identification and quantification respectively.

Synthetic Cannabinoids

Synthetic cannabinoids refer to substances with structural features that have enhanced affinities (K_1) for the cannabinioid receptors. This allows the drug to bind to one of the known cannabinoid receptors, i.e. CB_1 or CB_2, which are present in the human body (brains and organs). The synthetic cannabinoids, more correctly designated as cannabinoid receptor agonists that target the CB_1 receptor, thus mimic the effects of the psychoactive tetrahydrocannabinol (THC) present in cannabis plants. However many of these substances do not have structure that is chemically similar to THC.

These synthetic cannabinoids fall into six major groups as classified by the British Advisory Council on the Misuse of Drugs (ACMD) [1-2]. The classification of the synthetic cannabinoids is based on the chemical structures of the molecules. The six major groups are listed below:

(1) **Classical Cannabinoids** (THC, other constituents of cannabis: and their structurally related synthetic analogues e.g. HU-210, AM-906, AM-411, O-1184)
(2) **Nonclassical Cannabinoids** (cyclohexylphenols or 3-arylcyclohexanols such as CP-47,497-C8, CP-55,940, CP-55,244)
(3) **Hybrid Cannabinoids** (combinations of structural features of classical and non-classical cannabinoids, e.g. AM-4030)
(4) **Aminoalkylindoles (AAIs)**, which can be further divided into naphtoylindoles (e.g. JWH-018, JWH-073, JWH-398, JWH-015, JWH-122, JHW-210, JWH-081, JWH-200, WIN-55,212); phenylacetylindoles (e.g. JWH-250, JWH-251); naphtylmethylindoles and benzoylindolies (e.g. pravadoline, AM-694, RSC-4).
(5) **Eicosanoids** (endocannabinoids such as anandamide, and their synthetic analogues e.g. methanandamide)
(6) **Others**, diarylpyrazoles (selective CB1 antagonist Rimonabant), naptoylpyrroles (JWH-307), naphthylmethylindenes or derivatives of naphthalene-1-yl-(4-pentyloxynaphthalen-1-yl)methanone (CRA-13)

Some of the synthetic cannabinoid structures from the six major groups are listed in Figure 1.

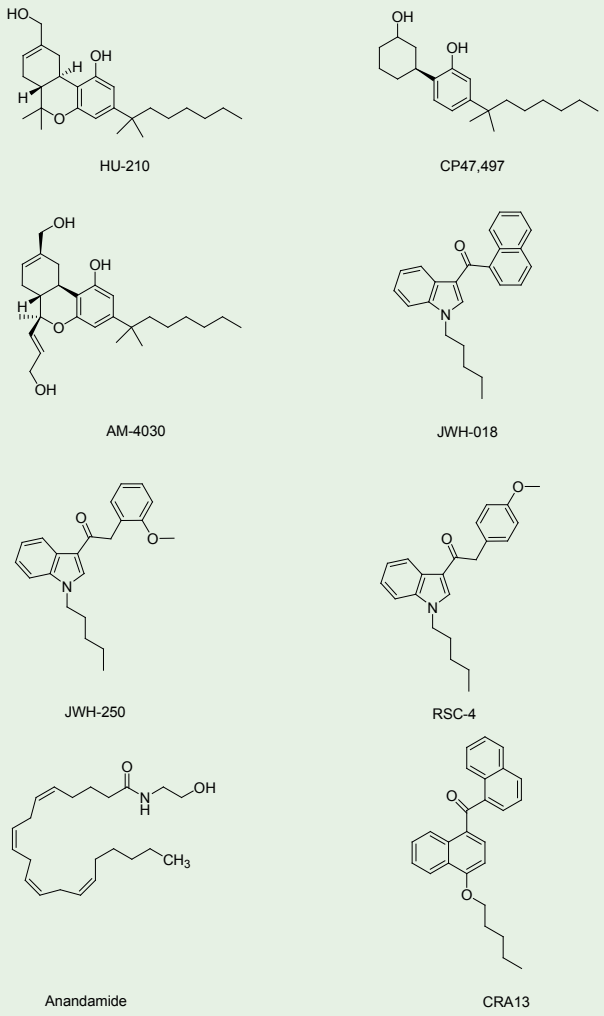


Figure 1: Structures of synthetic cannabinoids.

Synthetic Cathinones

Cathinone occurs naturally in the leaves of khat (*catha edulis*). It is similar in structure to amphetamine apart from the beta-ketone. Cathinone hence has effects similar to amphetamine. Starting from this cathinone structure, various modifications can be made to obtain derivatives of cathinone or synthetic cathinone such as [3-4]:

(1) Substitution at amine: Methcathinone or βk-methamphetamine, ephedrone or N-methylcathinone
(2) Substitution of atom at benzyl ring: the most prevalent of which appears to be mephedrone (4-MMC; 4-methylmethcathinone), 3-FMC
(3) Ring-substituted: methylone (βk-MDMA; 3,4-methylenedioxy-N-methylcathinone), MDPV (3,4-methylenedioxypyrovalerone), methedrone (βk-PMMA; 4-methoxymethcathinone) and PPP (α-pyrrolidinopropiophenone)

Synthetic cathinones are sold via the Internet as 'bath salts'. Some of the synthetic cathinone structures are listed in Figure 2.

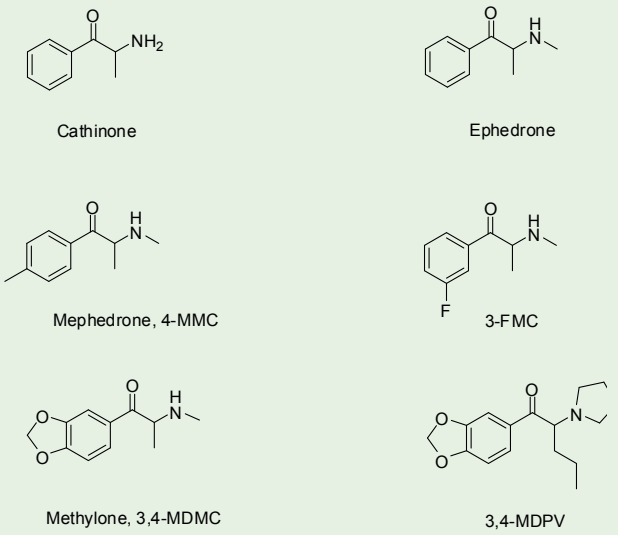


Figure 2: Structures of some common cathinone derivatives.

Ecstasy Tablets containing Legal Highs

From January to December 2012, our laboratory received approximately ten (10) and twenty (20) cases of Herbal products and Ecstasy tablets, respectively. However in this article, cases related to large seizure are highlighted which were analysed and found to contain a variety of legal highs. (Refer to Table 3). This paper presents the analytical results of the legal highs found in Herbal products and Ecstasy tablets.

Experimental Conditions

Herbal Products

Approximately 50 mg of the herbal products was placed in a 5 ml volumetric flask and 5 ml of methanol was added. This was sonicated for 10 min then left for 10 min to settle. The methanol extract was analysed by GC/MS and LC-MS/MS.

Gas Chromatography/Mass Spectrometry (GC/MS)

An Agilent 6890N GC equipped with an Agilent 5973 quadrupole mass-selective detector was used with parameters set as follows:

- Column: HP-5; 30 m x 0.25 mm i.d., 0.25 mm film thickness
- Carrier gas: Helium
- Injection mode: Splitless mode
- Injection volume: 1.0 µl
- Oven temperature: 80°C for 1 min, 10°C/min to 310°C & hold for 5 min
- Column flow rate: 0.7 ml/min
- Injector temperature: 200°C
- Detector temperature: 280°C
- Run time: 29.0 min

Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

The quantification of the synthetic cannabinoids present in these Herbal Products was performed using Waters Alliance 2695 equipped with a Onyx Monolithic C18 Column (Phenomenex), (2.00 i.d. x 50 mm). The instrument parameters were set as follows:

Liquid Chromatography (LC) Conditions

Liquid chromatography was used in a gradient elution;

- Column temperature: 30°C
- Flow rate: 0.3 ml/min
- Mobile phase A: 0.1% Formic Acid in water
- Mobile phase B: 0.1% Formic Acid in acetonitrile
- Gradient: 50% B increased to 80% B in the first 5 min
  80% B (isocratic for 3 min)
  50% B (equilibration for 2 min)
- Degasser: Normal
- Run time: 12 min
- Injection Volume: 10 µl

Mass Spectrometry (MS) Conditions

- MS system: Waters® Micromass® Quattro Micro™ or equivalent
- Ionization mode: Electrospray in positive mode (ESI +ve)
- MS/MS: Multiple Reactions Monitoring (MRM)
- Collision gas: Argon at a cell pressure of 3.6 x 10^{-3} mbar
- Source temperature: 130°C
- Desolvation temperature: 350°C
- Desolvation gas flow: 650 l/hr
- Cone gas flow: 20 l/hr
- Capillary voltage: 3.00 kV
- Cone voltage and collision energy as listed in Table 1

No.	Standards	Parent	Daughter	Cone Voltage (V)	Collision Energy (eV)
1	JWH-018	342.05	154.70 126.65	42.00 42.00	22.00 43.00
2	JWH-073	328.15	154.70 126.65	37.00 37.00	25.00 28.00
3	CP-47,497	319.15	301.20 233.05	12.00 12.00	8.00 15.00

Table 1: Cone voltage and collision energy for standards.

Ecstasy tablets

Approximately 50 mg of the ecstasy tablets was pulversied and placed in a 5 ml volumetric flask and 5 ml of methanol was added. This was sonicated for 10 min then left for 10 min to settle. The methanol extract was analysed by GC/MS and LC-MS/MS.

Gas Chromatography/Mass Spectrometry (GC/MS)

An Agilent 6890N GC equipped with an Agilent 5973 quadrupole mass-selective detector was used with parameters set as follows:

- Column: HP-5; 30 m x 0.25 mm i.d., 0.25 mm film thickness
- Carrier gas: Helium
- Injection mode: Split mode (~ ratio 40)
- Injection volume: 1.0 µl
- Oven temperature: 80°C for 3 min, 40°C/min to 300°C & hold for 25 min
- Column flow rate: 0.9 ml/min
- Injector temperature: 280°C
- Detector temperature: 280°C
- Run time: 33.5 min

Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

The quantification of the synthetic cathinones present in the Ecstacy Tablets was performed using Waters Alliance 2695 equipped with a Luna PFP Column (Phenomenex), (2.00 i.d. x 150 mm, 3 µm). The instrument parameters were set as follows:

Liquid Chromatography (LC) Conditions

Liquid chromatography was used in a gradient elution;

- Column temperature: 30°C
- Flow rate: 0.3 ml/min
- Mobile phase A: 10 mM Formate buffer
- Mobile phase B: Methanol
- Gradient: 60% A (Isocratic)
- Degasser: Normal
- Run time: 10 min
- Injection volume: 10 µl

Mass Spectrometry (MS) Conditions

- MS system: Waters® Micromass® Quattro Micro™ or equivalent
- Ionization mode: Electrospray in positive mode (ESI +ve)
- MS/MS: Multiple Reactions Monitoring (MRM)
- Collision gas: Argon at a cell pressure of 3.6 x 10^{-3} mbar
- Source temperature: 130°C
- Desolvation temperature: 350°C
- Desolvation gas flow: 650 l/hr
- Cone gas flow: 20 l/hr
- Capillary voltage: 3.00 kV
- Cone voltage and collision energy as listed in Table 2

No.	Standards	Parent	Daughter	Cone Voltage (V)	Collision Energy (eV)
1	Mephedrone (4-MMC)	178.05	160.02 145.00	22.00 22.00	12.00 20.00
2	Methedrone	194.10	161.00 145.95	22.00 22.00	20.00 28.00
3	Methylone (3,4-MDMC)	208.05	160.00 132.05	25.00 25.00	18.00 28.00

Table 2: Cone voltage and collision energy for standards.

Results and Discussion

The compounds detected in the Herbal Products and Ecstasy tablets via GC/MS and LC-MS/MS are tabulated in Table 3. A GC Chromatogram of a Herbal Product is given in Figure 3. GC Chromatograms of one type of Ecstasy tablet and a capsule are given in Figures 4 and 5 respectively. The structures and the mass spectra of these legal highs are given in Figures 1 to 2 and Figures 6 to 13 respectively. The MS of the compounds in samples were matched successfully with the spectrum in the SWGDRUG MS library (http://www.swgdrug.org) as well as that from literature [3].

The purity of legal highs present in these Herbal Products and Ecstasy tablets was determined to be between 0.2% – 24% (refer to Table 3). Analysis performed with GC/MS technique showed presence of synthetic cannabinoids in Ecstasy tablets. Due to that, the samples prepared for LC-MS/MS technique for analysis of synthetic cathinones were also analysed with LC-MS/MS technique for analysis of synthetic cannabinoids.

Reference materials are often not available particularly for newly emerging legal highs. When available for purchase, these reference materials could often be quite costly. In the LC-MS/MS technique, target compound quantification method was used and due to limitation in the standards, quantification was done only for one synthetic cannabinoid (JWH-018) and two synthetic cathinones (4-MMC and Methylone).

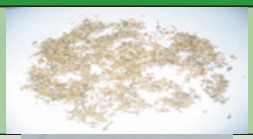
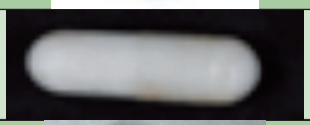
Image	Colour	Imprint	GC/MS Results	LC-MS/MS Results
	Green	-	JWH-250, JWH-018, AM-2201	0.2% JWH-018
	Beige	MITSUBISHI	3-FMC, 4-MMC, 3,4-MDPV, JWH-018, Caffeine	11% 4-MMC, 0.5% JWH-018
	Green	SUPERMAN	3,4-MDPV, JWH-018, Caffeine	0.4% JWH-018
	Grey	-	Methylone	24% Methylone
	Grey	-	3,4-MDPV, Caffeine	
	Brown	Star	4-MMC, Methylone 3,4-MDPV, JWH-081, Caffeine	2% 4-MMC, 8% Methylone
	Brown	-	4-MMC, Methylone 3,4-MDPV, JWH-081, Caffeine	3% 4-MMC, 9% Methylone

Table 3: Herbal Products and Ecstasy Tablets containing legal highs.

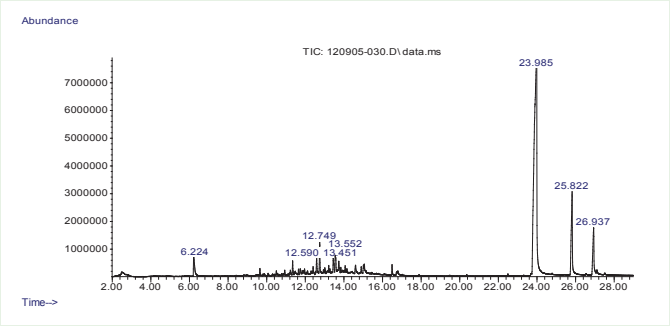


Figure 3: Expanded GC Chromatogram of the Herbal products.

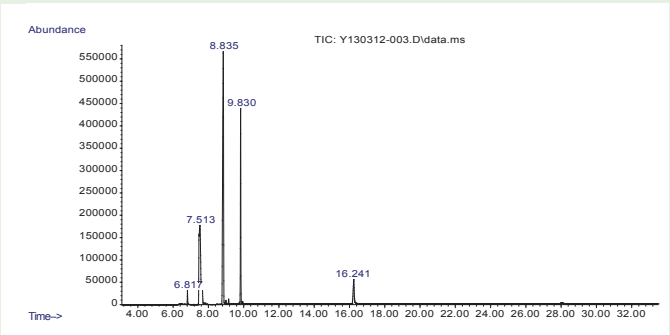


Figure 4: Expanded GC Chromatogram of the beige ecstasy tablet stamped with an imprint of a MITSUBISHI.

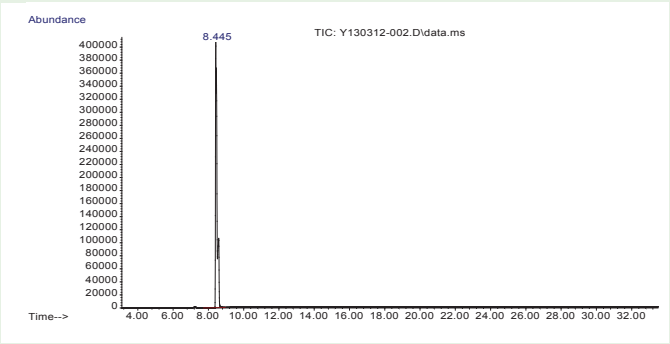


Figure 5: Expanded GC Chromatogram of the capsule which contains grey substances.

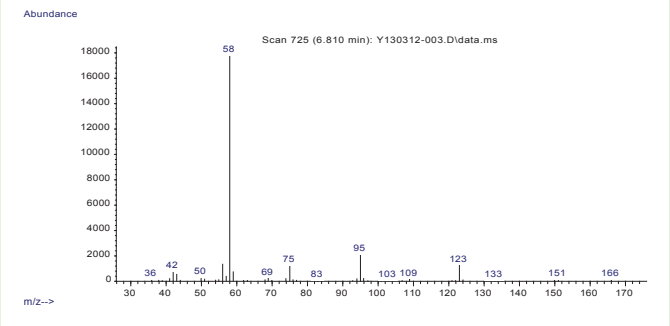


Figure 6: Mass Spectrum of 3-fluoromethcathinone (3-FMC).

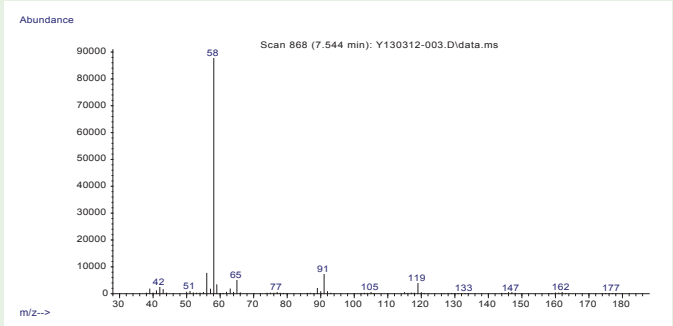


Figure 7: Mass Spectrum of 4-methylmethcathinone (4-MMC).

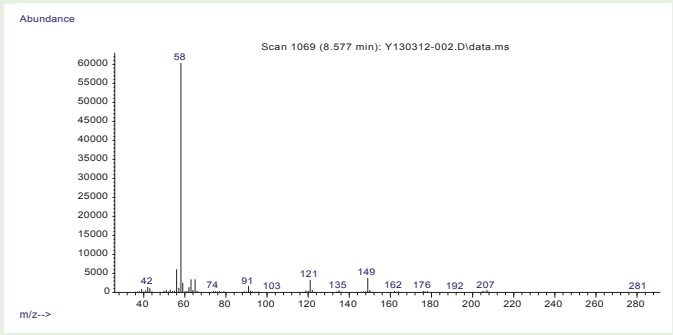


Figure 8: Mass Spectrum of 3,4-methylenedioxymethcathinone (Methylone).

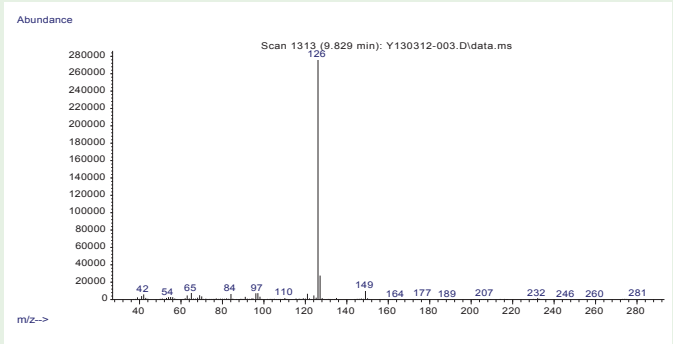


Figure 9: Mass Spectrum of suspected 3,4-methylenedioxypyrovalerone (3,4-MDPV).

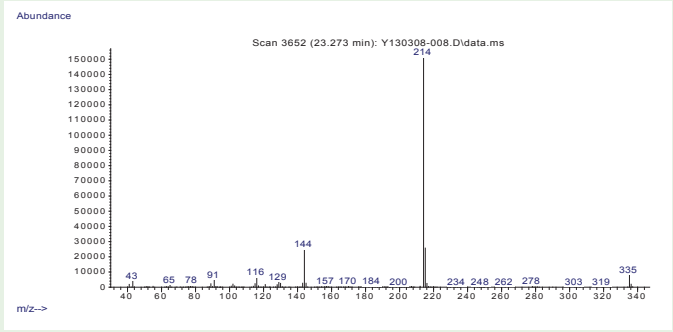


Figure 10: Mass Spectrum of JWH-250.

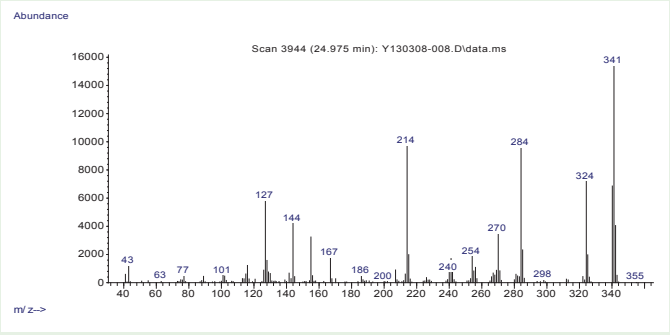


Figure 11: Mass Spectrum of JWH-018.

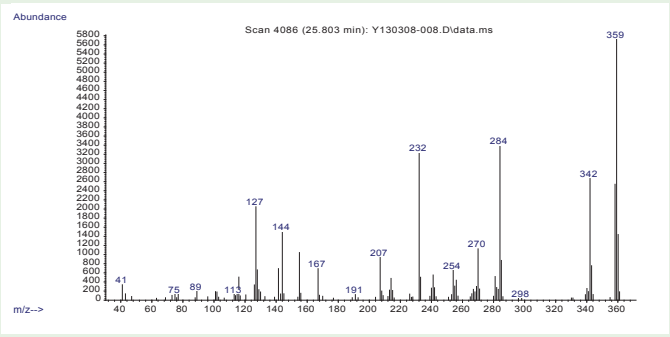


Figure 12: Mass Spectrum of AM-2201.

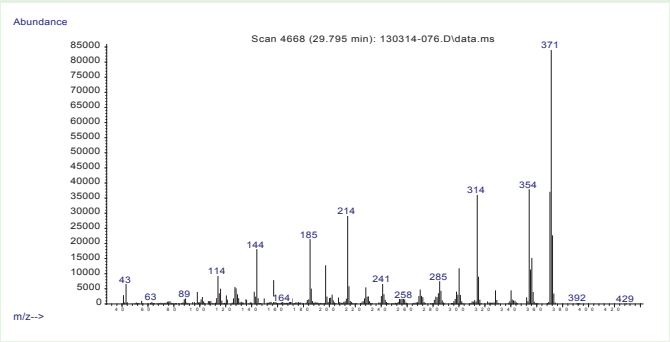


Figure 13: Mass Spectrum of JWH-081.

Conclusion

The findings from our analysis on some of the casework samples received in the laboratory showed the presence of this group of legal highs in the Herbal products: JWH-250, JWH-018 and AM-2201. The following compounds were found in the Ecstasy tablets: 3-fluoromethcathinone (3-FMC), 4-methylmethcathinone (4-MMC), 3,4-methylenedioxymethcathinone (Methylone), 3,4-methylenedioxypyrovalerone (MDPV), JWH-018 and JWH-081 (Refer to Table 3). It should be noted that synthetic cannabinoids are not only present in Herbal Products but also present in Ecstasy tablets. In this study, the presence of legal highs was indicated by the mass spectrometric data from SWDRUG website and literature. Legal highs are not controlled in both Dangerous Drugs Act 1952 and Poisons Act 1952 in Malaysia. The quantification by LC-MS/MS technique with target compound method has a limitation due to availability in wide range of standards. This technique however is not routinely available in most forensic laboratories. Current work is in progress in the laboratory to develop a HPLC method to cover the quantification of a wide range of legal highs. Legal highs are new designer drugs, therefore literature and studies containing detailed analytical data of such legal highs are often limited. In order to obtain better findings and conclusion in analysis, sharing of forensic data such as mass spectrometric library and chromatographic data would be very useful for all forensic drug testing laboratories.

References:

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4. Hudson S, Ramsey J. The emergence and analysis of cannabinoids. *Drug Testing and Analysis*. 2011;3:466-478.

Qualitative and Quantitative Analysis of Methcathinone by LC-MS/MS Method

Dr. Chang Ying, Mr. Zhang Chun Shui, Mr. Gao Li Sheng
Institute of Forensic Science, Ministry of Public Security, China

Abstract

Methcathinone was detected recently by the drug laboratory of the Institute of Forensic Science, Ministry of Public Security, People's Republic of China. The white crystalline substance was delivered to our laboratory by the police, where it was subsequently analysed by LC-MS/MS. This is the first report of finding this substance in Mainland China. It is suggested that more attention should be paid on cathinone family designer drugs in Asia.

Introduction

Methcathinone is a monoamine alkaloid and psychoactive stimulant similar to cathinone, the primary psychoactive compound in the khat plant (Figure 1). It is used as a recreational drug and is considered to be addictive [1-2]. It is usually snorted, but can be smoked, injected, or taken orally.

Methcathinone is listed as a Schedule I controlled substance by the United Nations Convention on Psychotropic Substances 1971.

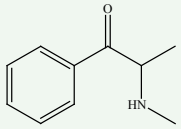

Figure 1: Methcathinone chemical structure.

Materials and Methods

Instrumentation

Agilent LC/QQQ: The liquid chromatographic system used belongs to the Agilent 1290 series and the MS system was an Agilent 6460 triple quadrupole mass spectrometer equipped with an electrospray interface operating in positive ionization mode.

Chemicals and reagents

Acetonitrile: Chromatographic grade, purchased from Thermo Fisher Scientific (American);

Formic acid: Chromatographic grade, purchased from Fluka (Switzerland);

Methcathinone standard was purchased from Cerilliant (American). Stock standard solution was prepared in methanol at concentration of 1 mg/ml and stored at 4°C. Analytical standard solution was prepared from above stock standard solution.

Chromatographic condition

Agilent Zorbax® Eclipse Plus C18 column (100 mm × 2.1 mm, 1.8 µm), mobile phase is 0.1% formic acid (FA) and acetonitrile; the gradient elution, flow rate is 0.3 ml/min. Injection volume is 3 µl.

Time (min)	0.1% FA (%)	Acetonitrile (%)
0	90	10
2.8	90	10
3.0	60	40
5.0	60	40
5.1	90	10

Table 1: Gradient elution.

MS condition

Mass spectrometry was carried out using an ESI source in positive ionization mode. Quantitative analysis was performed in multiple reaction monitoring mode. Nebulization gas (liquid nitrogen) pressure was 25 psi, Drying gas (liquid nitrogen) flow was 10 l/min, Drying gas temperature was 350°C, and ΔEMV was 400 V. Refer to Table 2 for details on precursor ion, product ion, fragmentor voltage, collision energy. For methcathinone standard's MRM and extract spectrum, see Figure 2.

Retention time	Precursor ion	Product ion	Fragmentor voltage (V)	Collision Energy (V)
2.210 min	164.2	131.1	85	20
	164.2	77.2	85	50

Table 2: Methcathinone's selected ion and optimised parameters.

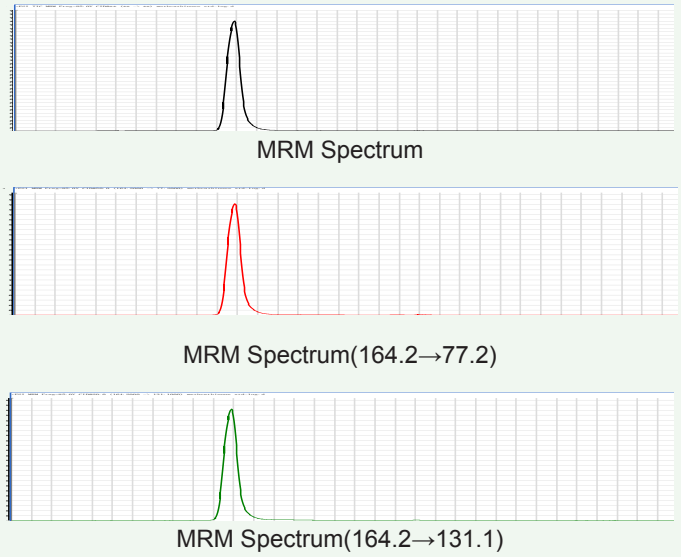

Figure 2: Methcathinone standard's MRM and extract spectrum.

Sample preparation

Approximately 10 µg of the white crystalline substance was dissolved in 10 ml methanol and then subjected to LC-MS/MS analysis. Injection volume is 3 µl.

Results and discussion

Calibration curve for methcathinone was linear from 1 to 1000 ng/ml (A = 1054.9c - 73.697, R^2 = 0.9999). The detection limit of methcathinone was 0.04 ng/ml (S/N ≥3). To establish the accuracy of the calibration curve, samples of low, medium and high concentrations of standard solutions were spiked with known amounts of methcathinone and measured by the above method. Methcathinone's recovery was 95.6% – 100.7%. The method proved to be precise for methcathinone, both in terms of intra-day and inter-day analysis, with coefficients of variation (CV) less than 5.28%.

This method was applied to a city Public Security Bureau sample, resulting in the same retention time and very similar Precursor ion-Product ion peak area ratios when compared to the methcathinone standard solution. By adopting this method, we are able to achieve qualitative determination of methcathionone. Using the external standard approach, the concentration of methcathinone was calculated as 25.4%.

Conclusion

This study established a LC-MS/MS qualitative and quantitative analysis method for methcathinone, using ultra-high performance liquid chromatography coupled with mass spectrometry MRM detection mode. The analysis time of this method is only 7 min. Compared with the traditional liquid chromatography, this method greatly shortens the analysis time and improves sensitivity. It provides a reliable analytical platform for the rapid analysis of samples.

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Introduction of a Quantitative Method for Stamp Examination

Dr. Han Xingzhou, Prof. Hao Hongguang, Mr. Wang Xiaoguang, Ms. Yan Haiqian, Dr. Qin Da, Mr. Ao Lin, Prof. Wang Jinsheng
Institute of Forensic Science, Ministry of Public Security, China

Abstract

This paper introduces the methods and research on quantitative features of stamp examination in China. Together with our collaborators, we have developed a software that can automatically compare two stamps and is able to choose up to 24 features to evaluate the likelihood ratio of the two stamps. Each feature is given a weight coefficient, and the total likelihood ratio can be accounted for. The results demonstrated that quantitative features can be helpful to the examiner in comparing stamps.

Introduction

Seals have existed for more than 3000 years in China, possessing historical significance of power, wealth and credit and today, they are regarded as artistic treasures of China. Seals also play a very important role in social life and economic work. Every government office, enterprise and individual has their own seal. The seal and the signature are both recognized legally in China.

Nowadays, stamp examination methods are well-established in China, but rely mainly on morphological examination. The first step is to determine the formation mode of the stamp. Most stamps look similar in red, blue or black, but not all are impressed directly by a seal; some stamps are forged through color photocopy, print or other printing methods. This first step of examination is very important as it can have a major influence on the final result.

Next, the questioned stamp is compared with reference samples. Usually, comparison is based on two aspects. One aspect is its specification features, which include content, size, shape and overall arrangement. Different materials, frequency of use and environment (temperature and humidity) can lead to different shrinkage change, therefore it is very important to examine the samples during the same period. Another aspect is the features, which are presented as special shape, including hand-carved features, wear features, character font, false proof seal features, *etc*. The stamp examination methods mainly include: (1) overlapping comparison; (2) drawing of lines to compare; (3) concatenation comparison; (4) measurement comparison; (5) detail features comparison.

Those methods are mainly based on morphology inspections. The examiner's skill and experience play an important role in the examination process. With the progress of science and technology, a challenge faced by the examiner is the need to improve the accuracy, scientificity and objective of the stamp examination method. For this reason, it was reported that similar research of stamps was conducted in Korea and some other countries. However, the literature survey failed to identify publications in this respect. In this paper we present a software system that evaluates 24 stamp features quantitatively, using sample for verification. In contrast to English or other languages, Chinese characters are contained within a framework of an "imaginary box". The seals are made up with a combination of different words, shapes or digits. The examination of seals are performed differently in other countries.

Materials and Methods

We have been conducting some research on quantitative features of stamp examination for many years. The system presented in this paper is based on a technology that measures digital surface topography of a 3D holographic image and wavelet transform to obtain the true shape and characters of each stamp, followed by the analysis of the likelihood ratio of two stamps. Due to the difference in language and examination methods, we chose different characteristics to evaluate stamps in this software. The system will compare the two stamps automatically and choose up to 24 features (named X_i, i = 1,2……24) to evaluate the likelihood ratio of the two stamps. Each feature was assigned different weight coefficient (a_i%, i = 1,2……24), after which the final likelihood ratio (A%) of the questioned stamp and the samples was obtained using following formula:

$$A\% = \sum_{i}^{24} (X_i * a_i\%)$$

Some examples of the 24 features are as follows: the average stamp surface overlap ratio, the frame overlap ratio (Figure 1a), the ring typesetting overlap ratio (Figure 1b), the Five-pointed star overlap ratio, the frame width, the angle (Figure 1c) and the distance (Figure 1d) of the Five-pointed star, the seal number overlap ratio, etc. The weight coefficient assigned to each feature is guided by a collective of researches. Some are given higher ratio, whereas some are given a lower ratio. In previous studies, 20 experts were invited to choose and give weight coefficients to each feature. The 24 features chosen and their corresponding weight coefficient are shown in Table 1. For illustration, we will explain how the weight coefficient of the frame overlap ratio (Figure 1a) is assigned as 10%. In previous studies, 20 experts separately gave a coefficient of the frame. One expert gave 6%, another gave 7%, the third gave 8%, the forth gave 10%, the fifth gave 12%, and so on. The average coefficient was calculated to be 9.8%, hence rounding up to 10% in the software. Moreover, we found that there was little difference in the final result if we changed the coefficient to 9% or 11%. The coefficient in the software could be modified by surveying more experts to give a coefficient.

a) The frame overlap ratio

b) Ring typesetting overlap ratio

c) The angle of the Five-pointed star

d) The distance of the Five-pointed star

Figure 1: Examples of the 24 features

Many confirmatory tests were performed to establish the software. To give an example, in one such test, we made a photosensitive seal, and impressed one stamp on a piece of paper, named K1 (Figure 2b). We made another photosensitive seal, using high-emulation technology to forge K1, and impressed one stamp on a piece of paper, named Q1 (Figure 2a). Q1 and K1 were then examined and compared by the software.

a) Q1 *b) K1*

Figure 2: Overall features of Q1 and K1

Results and Discussion

No.	Name	Weight coefficient	Value
1	Average stamp surface overlap ratio	20.00%	76.23%
2	The frame overlap ratio	10.00%	81.75%
3	Ring typesetting overlap ratio	20.00%	74.00%
4	Five-pointed star overlap ratio	3.00%	92.86%
5	Seal number overlap ratio	5.00%	73.72%
6	Straight typesetting	10.00%	79.88%
7	Frame size	8.00%	88.55%
8	Frame width	8.00%	93.45%
9	Five-pointed star distance	1.00%	90.07%
10	Five-pointed star valley distance	1.00%	96.80%
11	Five-pointed star angle	1.00%	100.00%
12	Five-pointed star valley angle	1.00%	92.92%
13	Height of the star horn	1.00%	95.76%
14	Width of the star horn	1.00%	91.80%
15	Ring typesetting frame	1.00%	99.46%
16	Ring typesetting shape	1.00%	99.27%
17	Ring typesetting position	1.00%	98.35%
18	Angle of ring typesetting	1.00%	90.82%
19	Seal number frame	1.00%	93.15%
20	Seal number shape	1.00%	96.58%
21	Seal number position	1.00%	96.71%
22	Straight typesetting frame	1.00%	98.99%
23	Straight typesetting shape	1.00%	99.68%
24	Straight typesetting position	1.00%	98.50%
			Total likelihood ratio: 82.63%

Table 1: Name, weight coefficient and measurement value.

The comparison result of Q1 and K1 is shown in Table 1. Twenty four features were chosen in the comparison and the total likelihood ratio of Q1 and K1 was calculated to be 82.63%. The experiment demonstrates that the system was very useful in identifying if the stamps were in the similar impress condition (the similar shades). According to 1875 groups of research data about photosensitive seals advanced, when A% was greater than 96.838%, the two stamps were very likely impressed by the same seal; when A% was less than 86.266%, the two stamps were very likely impressed by different seals; when A% was equal or greater than 86.266% and less than 96.838%, the result is inconclusive. This situation must be further examined by experts. Due to the above results, it can be concluded that Q1 and K1 were very likely impressed by different seals.

The results of this study demonstrated that quantitative features are very useful for examiners to express their views about the evidence, which is direct-viewing to directly shows higher and lower overlap ratio of the features and stamp details. In our daily work, we often use it to examine stamp cases, but just as a tool to aid the examiner. We cannot depend on it entirely. At the end of the examination, the examiner must give his/her opinion according to the results. In addition, the software has several limitations. Firstly, due to the fact that the features chosen and weight coefficients accounted for were obtained from only 20 experts, further research efforts are required to invite more experts to improve and perfect the weight coefficients, as well as to develop much more features to modify it. When we invite more than 1000 experts, the coefficients will be better. Secondly, when the stamps are not clear enough (i.e. the questioned stamp is not clear, whereas the samples are clear, or vice-versa), the result will not be good. We are unable to use the results in such situations. It is only suitable for use during conditions when the stamps are clear and in the similar impressing condition. Determining if these stamps are clear enough is one of the difficult tasks faced not only by the software but also the examiners.

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A Case of Identification for Tetramethylammonium Chloride in Biological Samples by Liquid Chromatography–Tandem Mass Spectrometry

Mrs. Zhang Leiping, Mrs. Wang Ruihua, Mrs. Luan Yujing, Mrs. Du Hongyan, Mr. Yu Zhongshan, Mrs. Wang Fanglin, Mr. He Yi, Mr. Cui Guanfeng
Institute of Forensic Science, Ministry of Public Security, China

Introduction

Tetramethylammonium chloride is one of the major industrial chemicals used. It belongs to the simplest quaternary ammonium salts, bearing only four methyl groups tetrahedrally attached to the central N (Figure 1). It is a very hygroscopic colourless solid that is soluble in water and polar organic solvents [1]. Tetramethylammonium chloride is used in processes such as hydrofracking. In the laboratory, it has fewer synthetic chemical applications than quaternary ammonium salts containing longer N-alkyl substituents, which are used extensively as phase-transfer catalysts [2]. Tetramethylammonium chloride is very toxic to organisms. LD_{50} = 50 mg/kg (rat, p.o.).

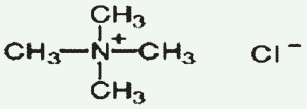

Figure 1: Tetramethylammonium chloride chemical structure.

This study reports one case involving tetramethylammonium chloride poisoning. A liquid chromatography-tandem mass spectrometry method was developed to detect and confirm this chemical in biological samples. Up to now, there has been no report of tetramethylammonium chloride determination in biological samples.

Case report

In this case which happened in the rural area of China, an 81-year-old man and a 78-year-old woman were found dead at home. There was evidence that the two people died within two hours after lunch. No evidence of any familiar poison was found and thorough examination of the bodies did not reveal any external injuries. The police reported that there was a water barrel at the scene.

The autopsy performed by local medical examiner was negative, and he could not specify the cause of death. Toxicological analyses were requested. Frozen samples of gastric contents, blood and the water barrel were submitted for toxicological analysis.

Materials and Methods

Instrumentation

LC was performed using a Shimadzu 30A system. Chromatography was achieved using ACQUITY UPLC® BEH HILIC (2.1 × 50 mm, 1.7 µm) eluted with a gradient delivery. An injection volume of 1 µl was used in all samples. The mobile phase consisted of two solvents; solvent A was 0.1% formic acid aqueous solution, and B was ACN. The gradient was programmed as follows: 0 – 0.1 min 90% B, 1.5 – 2.5 min 50% B, 2.6 – 4.5 min 90% B. The flow-rate was 0.4 ml/min.

The MS apparatus was a AB5500 mass detector with an ESI in the positive ionization mode. The ESI inlet conditions were as follows: ion source temperature, 600°C; capillary voltage, 5500 V; desolvation gas, 70 psi; cone gas, 8 psi. The scan mode measurement combined with multiple reaction monitoring (MRM) conditions was performed.

Compound	Parent ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (eV)
Tetramethylammonium chloride	74.1	58	107	28
		59	107	21

Table 1: Mass spectrometric parameters of tetramethylammonium chloride.

Chemicals and reagents

Acetonitrile, formic acid and methanol were HPLC grade (Merck, Darmstadt, Germany). Tetramethylammonium chloride standard was purchased from Sinopharm Chemical Reagent Limited Corporation (China). All other chemicals used in the experiments were of analytical grade. Stock standard solution was prepared in methanol at concentration of 1 mg/ml and stored at -20°C. Working standards were prepared from standard stock solutions by appropriate sequential dilutions with distilled water and stored at 4°C.

Sample preparation

One ml of sample blood was mixed with 3 ml of acetonitrile. The mixture was vortex-mixed for 30 s, sonicated for 10 min and centrifuged at 8000 rpm for 20 min. After the supernatant was filtered through Acrodisc GHP (0.22 µm pore size and 13 mm diameter, Waters), the filtrate was detected by LC-MS/MS.

Residue of water barrel and gastric contents were diluted with distilled water 100 times. The supernatant was filtered through Acrodisc GHP, the filtrate was detected by LC-MS/MS.

Results and discussion

Tetramethylammonium chloride is an ammonium salt. It has strong polarity and cannot be detected by GC and GC/MS. Therefore, we developed a new LC-MS/MS method of tetramethylammonium chloride.

Five different concentration levels of tetramethylammonium chloride were spiked at blank blood samples and then analysed. The calibration curve was linear in the range of 1 - 100 ng/ml, with correlation coefficients that were routinely greater than 0.993. Limit of detection (S/N = 3) and limit of quantitation (S/N = 10) were 0.5 ng/ml and 1 ng/ml respectively. The recovery, accuracy and precision data were tested at concentration levels of 5, 20, 100 ng/ml. The mean recovery was 86.4% with a coefficient of variation of 3.2%. The intra- and inter-day precisions were within 10% at three concentrations.

Since the procedure proved to be sensitive, selective and reproducible, the method developed was applied to this fatal case. The case is positive of tetramethylammonium chloride. We detected tetramethylammonium chloride from all samples. The LC-MS/MS chromatograms obtained from the extract of the deceased's blood samples are shown in Figure 2.

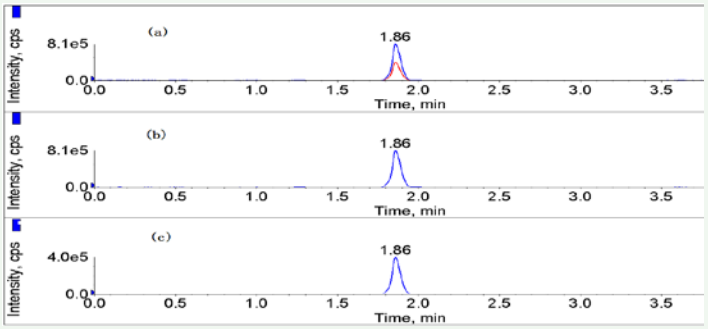

Figure 2: LC-MS/MS chromatograms of the deceased's blood samples. (a) XIC of MRM (two pairs) 74.1/58 (blue line) and 74.1/59 (red line); (b) XIC of MRM 74.1/58; (c) XIC of MRM 74.1/59.

Conclusion

The LC-MS/MS method showed to be appropriate for screening and identification of tetramethylammonium chloride in biological specimens. The autopsy and toxicological results led to the ruling, by the pathologist, that death was due to tetramethylammonium chloride intoxication. Local police investigated that the deceased had bought a chemical barrel for use as a water barrel, which was found at the scene. The cause of death was accidental poisoning of tetramethylammonium chloride.

References

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Determination of Chlorphenamine in Biological Samples by HPLC-MS

Mrs. Wang Ruihua, Dr. Luan Yujing, Mrs. Dong Ying, Dr. Du Hongyan, Mrs. Zhang Leiping, Mrs. Chang Jing, Mr. Yu Zhongshan, Mrs. He Yi
Institute of Forensic Science, Ministry of Public Security, China

Introduction

Chlorphenamine is used to help prevent allergic symptoms such as runny nose and sneezing. It can inhibit the central nervous system and has a sedative effect weaker than promethazine. People tend to have the symptoms of feebleness, dizziness, sleepiness and drowsiness after consumption; as such, criminals often make use of this drug to commit crimes. This paper reports a rapid and accurate method for the identification of chlorphenamine in biological samples.

Materials and Methods

Chemicals and standards

Chromatographic grade methanol was purchased from Merck (Darmstadt, Germany), and chlorphenamine maleate standard was purchased from Dr. Ehrenstorfer (Germany). Stock standard solution was prepared in methanol at concentration of 1 mg/ml and stored at 4°C. Analytical standard solution was prepared from the above stock standard solution. All the other chemicals used in the experiments were of analytical grade.

Sample preparation

To 2 g aliquot of liver sample homogenates, 1 M sodium hydroxide (100 µl) and water (1 ml) were added and mixed. The mixture was vortexed and extracted twice with ethyl acetate (6 ml) for 10 min. After centrifugation, the organic phase was removed and evaporated to dryness under a slow stream of nitrogen at 45°C. The extract was reconstituted in 0.5 ml 50:50 methanol/water and analysed.

Instrumentation

Separation was performed using a Shimadzu UHPLC LC-30A system coupled to an AB SCIEX QTRAP 5500 system. Sample volume of 5 µl was injected onto a Shim-pack XR-ODS column (2.1 x 50 mm, 2.2 µm). A gradient elution using water (with 0.1% ammonium acetate, mobile phase A) and methanol (aqueous mobile phase B) at a flow rate of 0.6 ml/min was shown in Table 1. Total run time was 3.5 min.

Time (min)	A%	B%
0	10	90
0.3	10	90
1.1	60	40
1.4	90	10
2.0	10	90
3.5	10	90

Table 1: Gradient profile

Mass spectra were obtained using a QTRAP 5500 system with electrospray ionisation (ESI). The mass spectrometer was operating in multiple reaction monitoring (MRM) mode. Sample introduction and ionisation was done in the positive ion mode. The declustering potential was 60 V. MRM transitions of m/z 275.0 → 230.3, 275.0 → 201.2 and 275.0 → 167.0 were monitored for chlorphenamine at collision energies of 20 V, 50 V and 55 V, respectively.

Results

The detection limit of chlorphenamine in liver sample was 1 ng/g (S/N = 3) and the lower limit of quantification (S/N = 10) was 5 ng/g. Analytical recovery was evaluated at the concentration level of 100 ng/g and determined by comparing the representative peak area of mass transition (m/z 275.0 → 230.3) of chlorphenamine extracted from spiked blank liver with the peak area of chlorphenamine standard at the same concentration. The mean recovery was 81% with a coefficient of variation of 4.8%.

Case Application

Case 1: A suspect asked a victim to come to his house, and made the victim drink the soup which was purportedly mixed with 2 tablets of chlorphenamine maleate. After the victim fell into deep sleep, the suspect kidnapped him and asked for his bank card and password. When the victim failed to meet the request, the suspect killed and buried the victim. Two months later, the suspect was arrested, the body was exhumed and the liver was taken for toxicological analysis.

Case 2: A suspect was acquainted with a victim from the internet, lured the victim to a hotel by reason of making friends, and made her drink the prepared inebriant containing chlorpheniramine maleate, then raped the victim while she fell into deep sleep. Two hours later, the victim woke up and cried for help. The blood of the victim was sampled by the police for toxicological analysis.

Applying the above method to the analyses of the two case samples, the results showed that chlorphenamine had not been detected in the first case, but was found to be present in the second case (Figure 1).

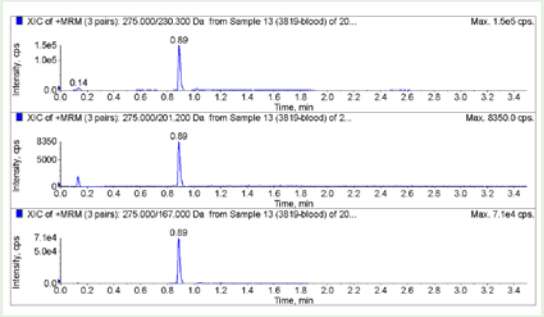

Figure 1: Extracted ion chromatogram of the blood sample in Case 2.

Discussion

The procedure described proved to be sensitive and reproducible, and can be used for the determination of chlorphenamine in poisoning cases. It can also be applied to the analysis of chlorphenamine in blood samples, as demonstrated in the second case. In Case 1 where chlorphenamine was not detected, it could have been due to the delay of the autopsy, which caused the decomposition of chlorphenamine by bacteria and enzymes in the decomposing liver.

Chlorphenamine has a weaker sedative effect than most hypnotic drugs, but the duration of the sedative effect is sufficient for criminals to commit crimes. Toxicology laboratories should include the screening of chlorphenamine in their test panels for specimens in drug-facilitated crimes.

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Determination of Sethoxydim in a Poisoning Case by HPLC-MS/MS

Dr. Luan Yujing, Mrs. Zhang Leiping, Mrs. Wang Ruihua, Mrs. Dong Ying, Dr. Du Hongyan, Mrs. Chang Jing, Mrs. Wang Fanglin, Mr. Yu Zhongshan, Mr. He Yi
Institute of Forensic Science, Ministry of Public Security, China

Introduction

Sethoxydim{(±)-2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio) propyl]-3-hydroxy-2-cyclohexen-1-one} belongs to a group of cyclohexanedione oxime herbicides. These herbicides are polar, nonvolatile, thermally labile, and applied at 100 - 300 g/ha to control the growth of annual and perennial grasses in dicotyledonous crops such as soybean, sugarbeet, and oilseed rape (Figure 1) [1].

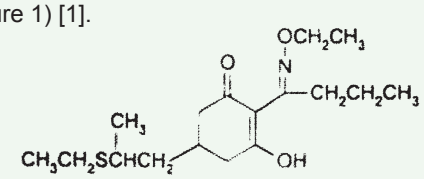

Figure 1: Chemical structure of sethoxydim.

Methods of analysis of sethoxydim using high performance liquid chromatography (HPLC) with a UV detector have had limited success, insufficient sensitivity and specificity due to the limited UV absorbance of sethoxydim. We therefore developed a specific and sensitive method using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Multi-residue methods have recently been reported for the determination of trace levels of herbicides in water and soil [2, 3]. However, there are no reports of determination of the levels of this herbicide in human samples. The aim of our work was to identify sethoxydim in gastric contents by LC-MS/MS in a case of suicide by taking sethoxydim.

Case history

In a case which happened in the rural area of northern China, a female was found with her body burned. She was sent to a hospital, but died later. Postmortem gastric contents were obtained for toxicological analysis.

Materials and Methods

Chemicals and standards

Chromatographic grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany), and sethoxydim standard was purchased from Dr. Ehrenstorfer (Germany). Blank human blood was supplied by Fuxing Hospital Affiliated to Capital Medical University (Beijing, China). Standard stock solutions containing 1 mg/ml of sethoxydim was prepared in distilled water and stored at -20°C. Working standards were prepared from standard stock solutions by appropriate sequential dilutions with distilled water and stored at 4°C. Calibrators and quality control (QC) samples for quantitative analysis were prepared by spiking these working standards in blank blood.

Extraction Procedure

The samples were prepared by adding working standard solutions (for calibrators and QC samples) or distilled water (for gastric contents). For qualitative analysis, 4 ml of acetonitrile was added to 1 ml of the samples. The mixture was shaken for 1 min with a vortex mixer and was centrifuged (10,000 × g, 10 min, 4°C). A 10 µl aliquot was used for the analysis.

Instrumentation

The analysis of sethoxydim was performed on Finnigan Surveyor LCQ Advantage MAX (Thermo Fisher Scientific, USA). Separation was performed with a XBridge RP C18 column (2.1 × 100 mm, 3.5 µm), which was maintained at 35°C. The mobile phase, consisting of 5 mM ammonium acetate-acetonitrile (50:50), was delivered at a flow rate of 0.2 ml/min. Detection was performed on a tandem mass spectrometry with electrospray ionisation source in positive ion mode. Sethoxydim was identified in full scan mode. The mass transition used for monitoring and quantification of sethoxydim was m/z 328.1 → 282.0, in matrix-matched standard solution. Parameters were as follows: capillary voltage was set to 29 V, spray voltage to 5.0 kV and the capillary temperature to 285°C. Nitrogen, obtained from a nitrogen generator (99.93%) was used for desolvation. MS data was collected in product ion scan mode.

Results and Discussion

To determine linearity, blank blood samples were spiked at ten different concentration levels and then analysed. The calibration curves were linear in the range of 10 - 500 ng/ml for sethoxydim, with correlation coefficients that were routinely greater than 0.998 in all cases. Limit of detection (S/N = 3) and limit of quantitation (S/N = 10) were 0.1 ng/ml and 0.5 ng/ml respectively. The recovery and precision data were tested at concentration levels of 10, 50 and 500 ng/ml. The mean recovery was 84.2% with a coefficient of variation of 2.1%. The intra- and inter-day precisions were within 10.5% at the three concentrations.

Since the procedure proved to be sensitive and reproducible, the method developed was applied to the fatal case presented. The LC-MS/MS results showed the presence of sethoxydim in the stomach contents. The LC-MS/MS chromatogram and MS/MS spectra obtained from the extract of the deceased's stomach contents are shown in Figure 2.

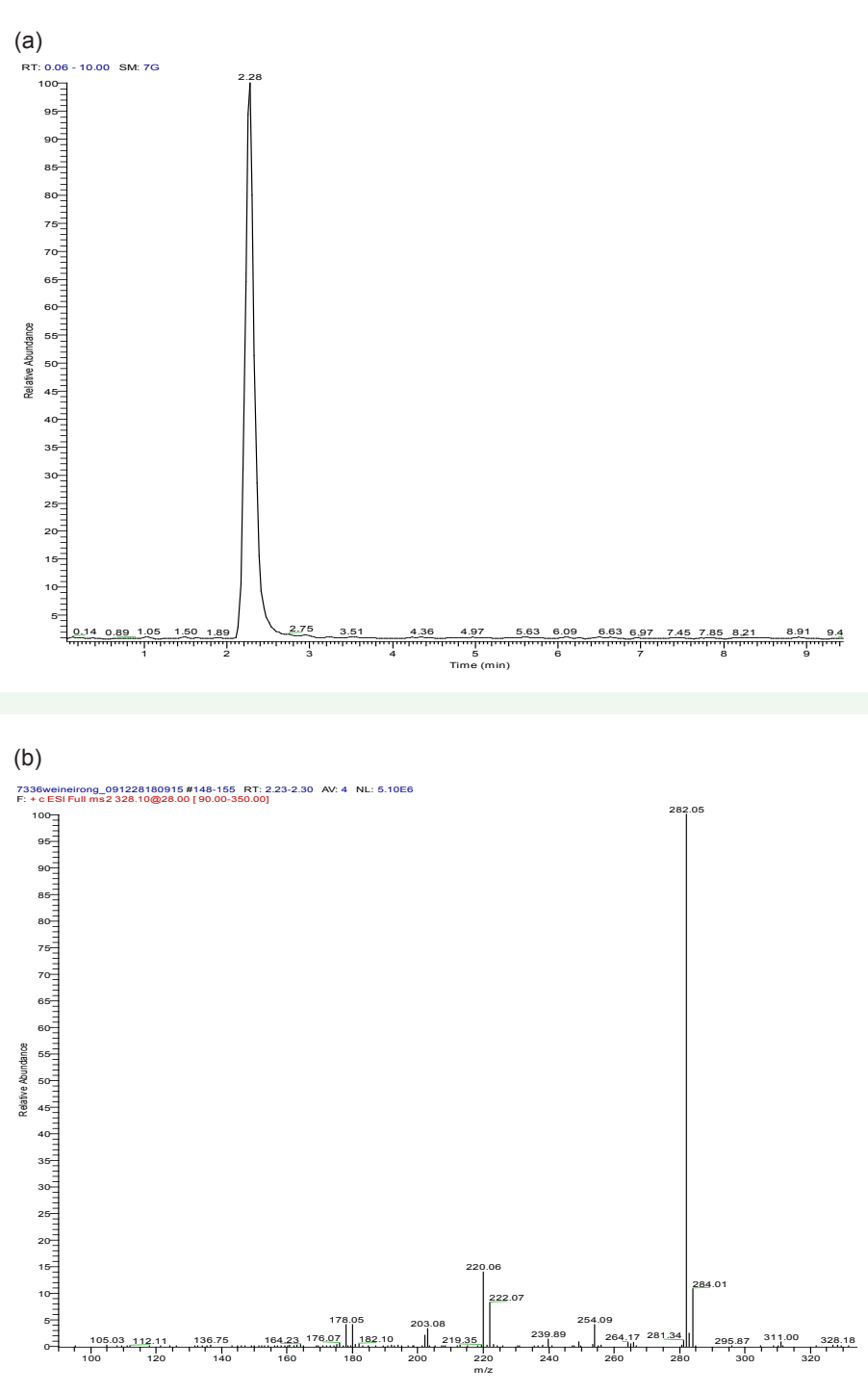

Figure 2: LC-MS/MS analysis of the deceased's stomach contents. (a) Total ion chromatogram and (b) mass chromatogram of m/z 282 in the product ion scan mode using 328.

Conclusion

The HPLC-MS/MS method described in this article was shown to be appropriate for screening and identification of sethoxydim in biological specimens. The autopsy and toxicological results led the pathologist to rule that death was due to sethoxydim intoxication. The intent of the decedent to take an overdose of sethoxydim was apparent from a plastic bottle found at the scene. The manner of death was determined to be suicide.

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Determination of Toxicants in Pepper Spray

Dr. Vu Cong Sau, Dr. Nguyen Van Ha
Institute of Forensic Science, Vietnam

Abstract

Three substances, 2-chlorobenzylidenemalononitrile, capsaicin and methanol, in pepper sprays were analysed by gas chromatography mass spectrometry with mass scanning conditions ranging from 10 to 500 and zero min delay solvent.

Introduction

There have been several cases of robbery where the criminals attack their victims with pepper spray to avoid pursuit. When the victim is attacked with pepper spray, it is harmful to the eyes. From the analysis of pepper sprays in this study, the substances 2-chlorobenzylidenemalononitrile, capsaicin and methanol were found.

Methanol

Other names of methanol are hydroxymethane, methyl alcohol, methyl hydrate, methyl hydroxide and methyl alcohol. CAS number 67-56-1, molecular formula CH_4O, molar mass 32.04 g/mol, boiling point 64.7°C.

Methanol has a high toxicity in humans. It is harmful to the central nervous system, and may cause blindness, coma, and death.

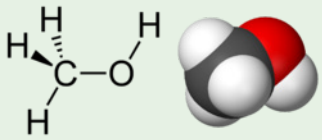

Figure 1: The molecular structure and molecular modelling of Methanol

2-chlorobenzylidenemalononitrile

IUPAC Name: (2-chlorobenzylidene) propanedinitrile; Other names: CS; Molecular formula: $C_{10}H_5ClN_2$; Molecular weight: 188.613 ± 0.011 g/mol, CAS number: 2698-41-1, white crystalline powder, characteristic odour; melting point: 96°C; boiling point: 315°C; elemental composition: C 63.68%, H 2.67%, Cl 18.8%, N 14.85%.

CS tastes bitter and causes watery eyes and burning sensation. CS is commonly used in pepper sprays.

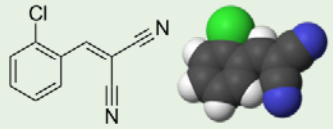

Figure 2: The molecular structure and molecular modelling of 2-chlorobenzylidenemalononitrile

Capsaicin

IUPAC Name: (E)-N-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide; CAS number: 404-86-4; Molecular formula: $C_{18}H_{27}NO_3$; Molecular weight: 305.4119 ± 0.0174 g/mol; elemental composition: C 70.79%, H 8.91%, N 4.59%, O 15.72%; melting point: 65°C; boiling point: 210 - 220°C; slightly soluble in water, dissolve well in ethanol, ether benzene, and chloroform.

Capsaicin causes skin irritation.

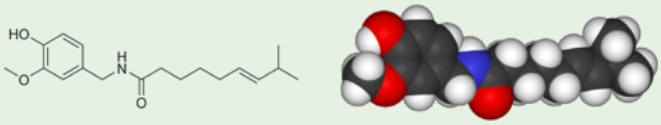

Figure 3: The molecular structure and molecular modelling of Capsaicin

Methods

Sample preparation

The sample is injected directly into the gas chromatography mass spectrometry (GC/MS)-HP Agilent Technologies 6890 N, MS 5973; Column DB1 Dimethyl polysiloxane, capillary: 30.0 m x 250 µm x 0.25 µm nominal.

Running mode GC/MS:

- Injection volume: 1 µl
- Injector temperature: 250°C
- Inlets: split ratio 20:1
- Flow: 0.6 ml/min
- Oven: 50°C (3 min), 15°C/min, 295°C (5 min)
- Detector: MS, scanning mass range (10 - 500)

Results and Discussion

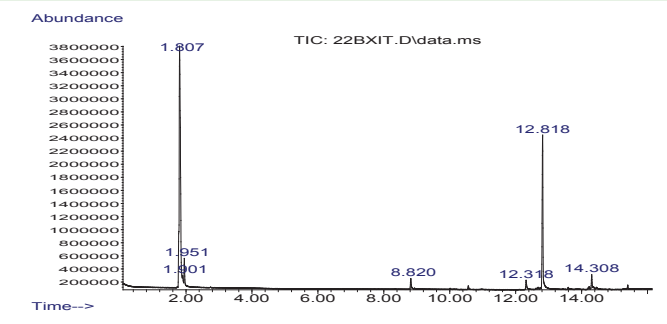

Figure 4: TIC of the substances in pepper spray

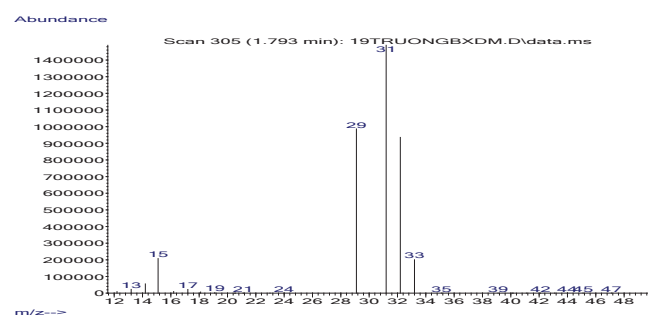

Figure 5: The mass spectrum of the peak with retention time of 1.7 min

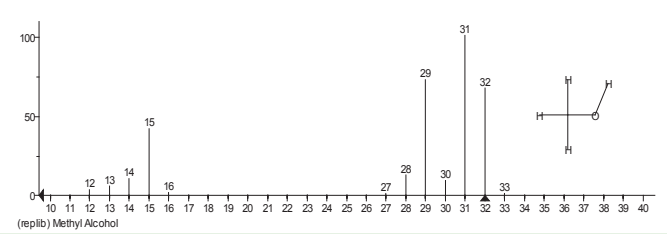

Figure 6: The mass spectrum of standard methanol

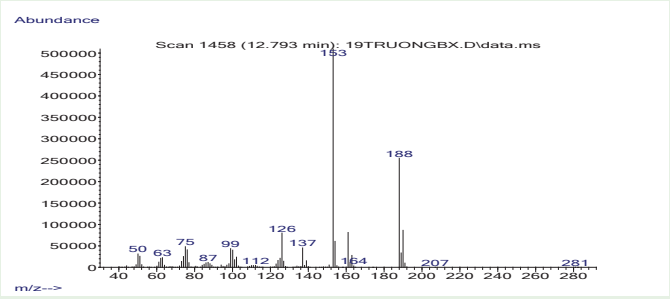

Figure 7: The mass spectrum of the peak with retention time of 12.7 min

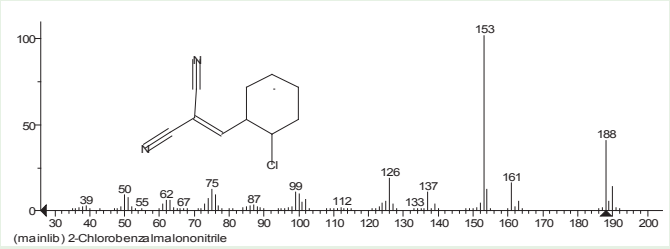

Figure 8: The mass spectrum of standard CS

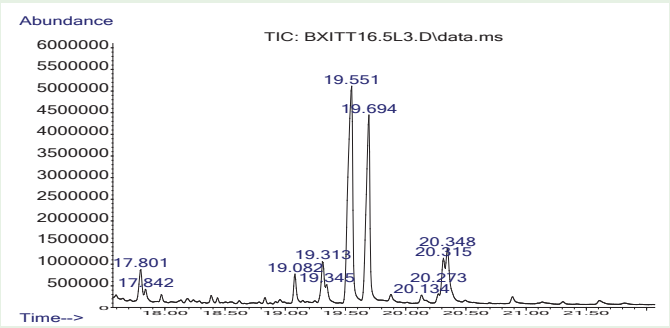

Figure 9: TIC of the substances in pepper spray

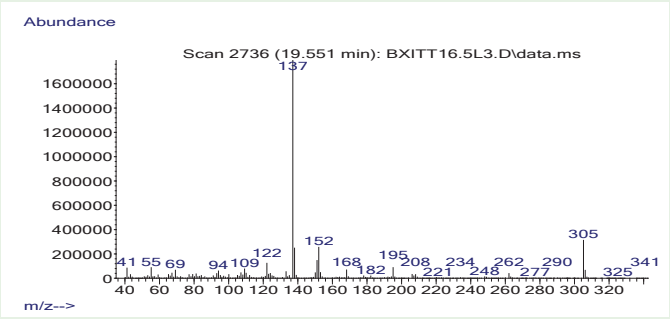

Figure 10: The mass spectrum of the peak with retention time of 19.5 min

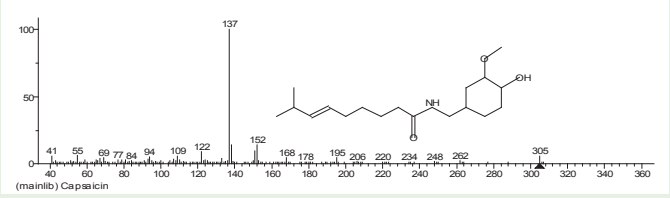

Figure 11: The mass spectrum of standard Capsaicin

The sample was analysed by GC/MS. From the result for TIC (Figure 4), 2 peaks at 1.7 min and 12.7 min were detected. The first peak for mass spectrometry (Figure 5) is equivalent to the standard methanol spectrum (Figure 6). The second peak for mass spectrometry (Figure 7) is equivalent to the standard CS mass spectrum (Figure 8). The peak at 19.5 min in TIC (Figure 9) for mass spectrometry (Figure 10) is equivalent to the standard Capsaicin mass spectrum (Figure 11). Hence, the pepper spray solvent was determined to contain methanol, capsaicin and CS.

Conclusion

According to the analysis of the pepper sprays by gas chromatography-mass spectrometry, methanol, capsaicin and CS were found. Mass spectrometry mode is set from 10 to 500; also as usual, if MS is set from 40 to 500, methanol will not be detected. Based on the analysis results above, which determined the toxicity of aerosols, the local authorities issued strict regulations for the production of pepper spray.

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First Reported Case of Ethyl Chloride Abuse in Singapore

Ms. Leong Hsiao Tung, Dr. Yao Yi Ju
Health Sciences Authority, Singapore

Introduction

Ethyl chloride (or chloroethane) is a colourless, flammable gas at ambient temperature and pressure. It is typically supplied in spray canisters and acts as mild topical anaesthetic for temporary pain relief when sprayed on skin. It has also been reported to be used as a recreational inhalant drug. A few fatal cases of ethyl chloride abuse have been reported [1-3]. Modes of ethyl chloride abuse include direct inhalation from a container, from a soaked or sprayed towel or from a plastic bag.

Case History

In August 2012, an 18-year-old female was found dead lying face down on her bed with her right hand clutching a "Ethylchloride" blue/white 100 ml spray can and her left hand clenching a plastic bag. There were no apparent signs of violence at the scene. The deceased's peripheral blood (oxalated), peripheral blood (plain), bile, vitreous humour, stomach contents, nasal swab and lung tissue were submitted to the laboratory for toxicological analysis. Routine analyses of volatiles in the blood, vitreous humour, stomach contents and lung tissues gave rise to an unknown peak which was later confirmed to be ethyl chloride.

Experimental

Materials

All reagents were of analytical grade. Ethyl chloride (1 mg/ml in methanol) was purchased from Supelco (Pennsylvania, USA). Methanol was purchased from RCI Labscan Limited (Bangkok, Thailand). N-propanol (used as internal standard) was purchased from BDH limited (England). An aqueous 12.5 mg/dl n-propanol solution was prepared by diluting appropriate amount of the standard with distilled water.

Preparation of calibration standards

As the stock solution for ethyl chloride was purchased as a 1 mg/ml methanolic solution, ethyl chloride working standards were prepared by diluting an appropriate volume of this stock solution in methanol. Working standards containing 1 mg/dl (or mg/100 ml), 2 mg/dl, 5 mg/dl, 10 mg/dl, 15 mg/dl and 20 mg/dl of ethyl chloride in methanol were prepared. Attempts to prepare the working standards in water were unsuccessful due to the poor solubility of ethyl chloride in water, which resulted in poor reproducibility of the instrumental response.

For the quantification of ethyl chloride in blood, calibration standards with concentrations 5, 10, 15 and 20 mg/dl were prepared by adding 0.05 ml of the above ethyl chloride working standards to 0.05 ml of blank blood in 20 ml crimp-capped headspace vials, together with 0.8 ml of 12.5 mg/dl aqueous n-propanol (IS) solution. In consideration of the presence of methanol in each calibration standard which will contribute to the vapour pressure, an equal amount of methanol was added to the headspace vial of each sample to give similar vapour pressure as the calibration standards. Hence for the preparation of the samples, aliquots of 0.05 ml blood sample, 0.05 ml of methanol, and 0.8 ml of IS were added to a 20 ml headspace vial.

Similar procedures were used for the preparation of aqueous calibration standards (1, 2 and 5 mg/dl) for the quantitation of ethyl chloride in vitreous humour.

Operating parameters

The samples were first screened for alcohols and volatiles on an Agilent G1888 headspace sampler coupled to an Agilent 6890N GC/FID equipped with a 5% Carbowax 20M on 100/120 Carbopack B (6 ft x 2 mm i.d. glass column). The temperatures were: headspace oven, 60 °C; injector and detector temperatures: 150°C. The GC oven temperature was set at 95 °C for the first 3.5 min and then ramped at a rate of 50°C/min to 150°C where it was held for 6 min. The vials were equilibrated for 18 min before injection. Injection time was 1 min and carrier gas flow was about 22 ml/min. Under these conditions, ethyl chloride and n-propanol (IS) eluted at 0.877 and 2.695 min, respectively, as shown in Figure 1.

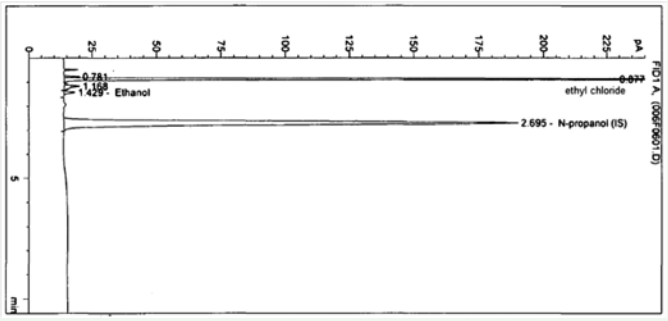

Figure 1: Elution of ethyl chloride in a 5% Carbowax 20M on 100/120 Carbopack B (6 ft x 2 mm i.d.) glass column.

Quantitation and confirmation of ethyl chloride was carried out using a different column (Agilent DB-ALC1 column, 30 m x 0.535 mm i.d. x 3.0 µm) in an Agilent 7890A GC/FID system coupled to an Agilent 7679A headspace sampler. The temperatures were: headspace oven 60°C; GC oven 35°C (isothermal), injector 150 °C and detector 200°C. The vials were equilibrated for 18 min before injection. The injection was made in 3 s with a split ratio of 5:1. Carrier gas flow was 6 ml/min. Under these conditions, ethyl chloride and n-propanol (IS) eluted at 2.166 and 3.894 min, respectively, as shown in Figure 2.

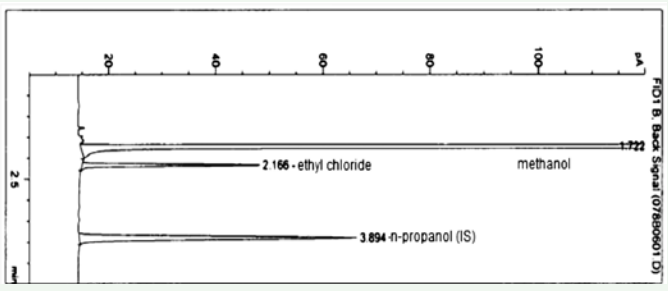

Figure 2: Elution of ethyl chloride and methanol in a DB-ALC1 (30 m x 0.53 mm i.d. x 3.0 µm) column.

Results and Discussion

Headspace GC analysis for volatiles in blood was performed using two different systems. There have been reports concerning the co-elution of ethyl chloride with ethanol in some systems [4, 5]. Our screening method using the 5% Carbowax 20M packed column did not have that problem (see Figure 1). However, it was found that methanol (retention time 0.970 min) eluted very closely to ethyl chloride and hence both volatiles were not well resolved on this column. This poses a problem to quantitation as the ethyl chloride working standards were prepared in methanol. The problem was resolved by the use of DB-ALC1 where methanol was well resolved from ethyl chloride (see Figure 2).

Ethyl chloride calibration standards were linear with R^2 greater than 0.993. The results of the analyses in the various specimens are shown in Table 1.

	Peripheral blood (Oxalated)	Vitreous humour	Stomach contents	Lung
Ethyl Chloride	6.2 mg/dl	<1 mg/dl	Detected	Detected

Table 1: Results of ethyl chloride in the various specimens.

Concentrations of ethyl chloride in the peripheral blood and vitreous humour of the deceased reported in this case were much lower than the fatal levels reported in other literatures [1, 2]. This might be due to a loss of ethyl chloride during storage as it is a highly volatile solvent.

Conclusion

Although there have been news reports on abuse of ethyl chloride spray in Singapore in 2009 [6], this was the first case of ethyl chloride being detected in biological specimens. A month after this case, there was another case of suspected ethyl chloride abuse by a 25-year old female who was admitted to the hospital twice. Her blood samples were found to be positive for ethyl chloride on both occasions, with levels lower than that reported in this post-mortem case. The method described here has been shown to be suitable for the detection and quantitation of ethyl chloride in biological specimens.

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The Elimination of Interference from Blood Contamination in Paint Analysis in a Hit-and-run Accident Investigation: A Case Report

Mr. Sun Zhenwen, Mr. Quan Yangke, Mr. Sun Yuyou, Mr. Tao Keming
Institute of Forensic Science, Ministry of Public Security, China

Abstract

Paint evidence contaminated with bloodstain in a hit-and-run accident was analysed. Purification with ethanol, and infrared spectral subtraction were methods used to reduce or eliminate interference effects due to the presence of blood, and thus enhancing the reliability of paint comparison results.

Introduction

Paint evidence is widely encountered in crime scenes associated with hit-and-run accidents, robberies and burglaries [1]. Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) are the most frequently used methods to distinguish paints in forensic examination [2, 3]. Unfortunately, contamination of paint samples by environmental substances is a technical challenge often encountered in actual forensic casework. In order to reach reliable conclusions, the forensic paint examiner should take some countermeasures to mitigate these interferences.

This paper presents a case study on the examination of smeared paint samples on a hit-and-run victim's clothing and comparison with known paints from two suspect trucks in order to determine whether they could have shared the same origin.

Background of the Case

A person was hit by a truck and died on the spot. The driver continued driving and fled from the accident scene. Green paint smears were recovered from two areas of the victim's clothing (designated as "Sample 1a" and "Sample 1b", Figure 1). There were no witnesses and surveillance videos available at the scene, thus the police could only investigate the accident through surveillance videos collected from other crossings nearby. Finally, two suspect trucks (A and B) were found and known paint samples were collected from them for comparison (designated as "Sample 2" and "Sample 3" respectively).

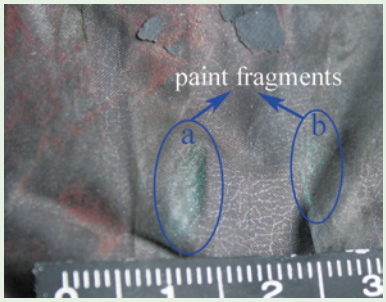

Figure 1: Green paint smears on the victim's clothing.

Methods

Microscopic examination of physical features of the paint samples was performed using a stereomicroscope. The organic components and elemental compositions were then analysed by FT–IR and SEM/EDS respectively.

Results and Discussion

Under stereomicroscope observation, bloodstains (red stains in the left area of Figure 1) were found around Area "a" of the victim's clothing. In order to determine the chemical component of the paint samples, Sample 1a and Sample 1b from the victim's clothing were compared with Sample 2 from Truck A, and Sample 3 from Truck B.

FT–IR spectra of the samples are presented in Figure 2a - 2d. Alkyd resin (peaks at 743, 1071, 1121, 1263 and 1728 cm^{-1}) and $CaCO_3$ (peak at 876 cm^{-1}) were found in all the samples [1]. There were some spectral differences between Sample 1a and Sample 2. However, Sample 1b and Sample 2 had no essential differences. The spectrum of Sample 1a had an absorption peak at 1658 cm^{-1} which was not present in Sample 1b. FT-IR results therefore indicated either of two possibilities: (I) the paint smears on the victim's clothing might be of two different kinds of paint; or (II) Sample 1a might have been contaminated by other substances. In order to verify the difference between Sample 1a and Sample 1b or Sample 2, Sample 1a was purified with ethanol and further examinations were performed. After purification, the absorption peaks at 876 and 1658 cm^{-1} were reduced (Figure 3a), thus confirming the existence of contamination which could not be completely eliminated by ethanol purification.

Contamination by blood components around Area "a" was not discernible based on visual inspection. However, assuming that Sample 1a could have nonetheless been contaminated with blood traces, we recorded the IR spectrum of a bloodstain (Figure 3b). It showed strong bands at 1652, 1544 and 1240 cm^{-1} which were assigned to amide I, amide II and amide III, respectively, the typical absorption bands of protein [4]. After software-enabled subtraction of the blood spectrum, the subtracted spectrum of Sample 1a (Figure 3c) showed no essential difference from the spectra of Sample 1b and Sample 2. Our results therefore demonstrated that the paint smears collected from Areas "a" and "b" on the victim's clothing were indeed the same kind of paint as Sample 2.

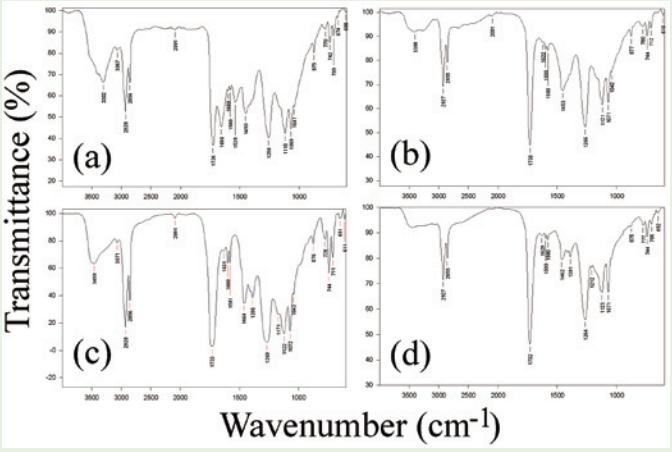

Figure 2: FT-IR spectra of (a) Sample 1a (before purification with ethanol), (b) Sample 1b, (c) Sample 2, (d) Sample 3.

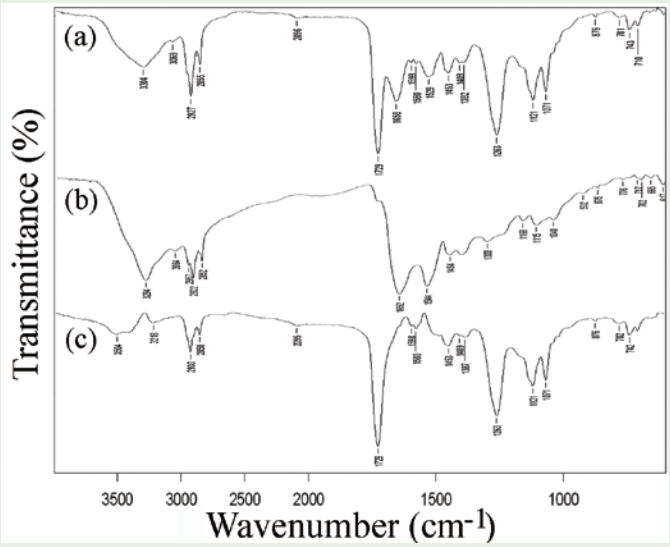

Figure 3: FT-IR spectra of (a) Sample 1a (after purification with ethanol), (b) bloodstain, (c) Sample 1a (after spectral subtraction).

In order to determine whether Sample 1 was contaminated by environmental substances, the pigment particles were selected for analysis by SEM/EDS. Comparison of EDS results showed that Sample 1 was indistinguishable from Sample 2, but could be distinguished from Sample 3 by the absence of Ti (Figures 4a - 4c).

According to the results of above-mentioned examination and analysis procedures, the paint smears on the clothing of the victim could have originated from suspect truck A, and could not have originated from suspect truck B.

Conclusion

In this paper, we demonstrated that the interference effects due to contamination of paint smears by blood were reduced but not completely eliminated by purification with ethanol. The interference effects were completely eliminated by infrared spectral subtraction; this permitted the correct determination of chemical components in the paint smears, and accurate comparison with the known samples. This case study provides a reference for paint evidence analysis in similar situations.

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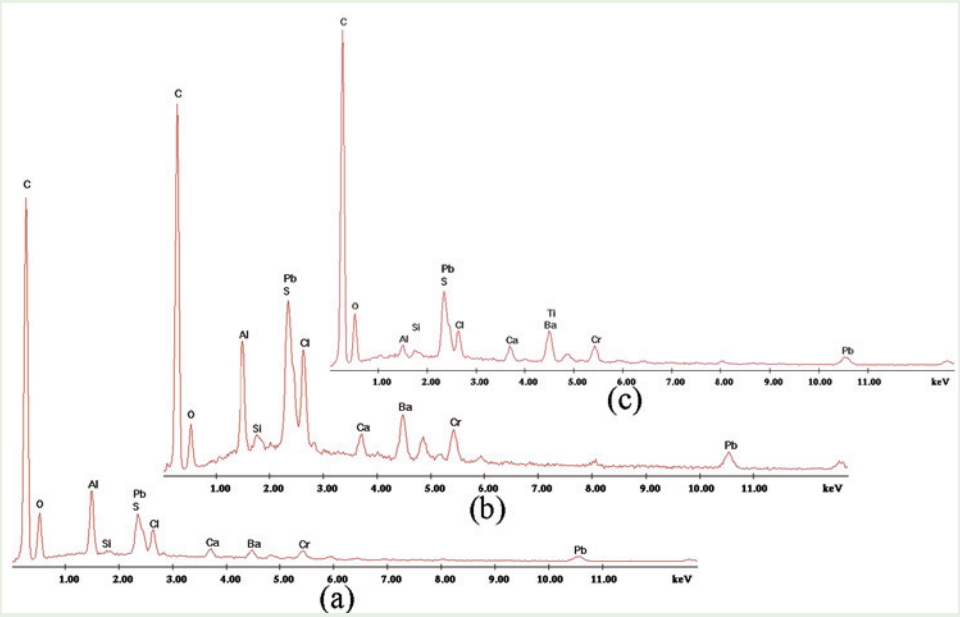

Figure 4: SEM/EDS spectra of pigments: (a) Sample 1, (b) Sample 2, (c) Sample 3.

New Insights Of An "Old" Evidence Type: Plastic Bags

Ms. Jean Chen Xiuxiu, Ms. Lim Shing Min, Ms. Vicky Chow Yuen San, Dr. Alaric Koh Chin Wai, Ms. Oh Suat Ping, Ms. Lim Chin Chin
Health Sciences Authority, Singapore

Background

Drug packaging materials such as plastic bags are often submitted to the Forensic Chemistry and Physics laboratory (FCPL) of the Health Sciences Authority (HSA) by the Central Narcotics Bureau (CNB) of Singapore for forensic analysis. Amongst these packaging materials, plastic bags are commonly used and have proven to be very useful in providing links between the drug traffickers and consumers of drugs. While the examination of a variety of plastic bags has been studied and documented in forensic literature, these studies focused on the applicable examination techniques and their usefulness. The variations in physical characteristics (weight, dimensions, thickness and polarising patterns) present within sequences of consecutively manufactured bags are currently not found in forensic literature. Understanding such variations is crucial in cases where there are no consecutively matching bags through manufacturing marks examination. The findings from this study will assist in determining if such bags could be associated with each other. Furthermore, there is little literature available for snap-lock bags which are frequently used in the drug scene in Singapore. Hence, snap-lock bags will be the focus in this study.

Objective

The objective of this paper is to study the physical characteristics of sequences of consecutively manufactured snap-lock bags of a particular size that is commonly encountered in casework so as to establish the range of acceptable internal variation for each physical characteristic.

Materials

Ten packets of snap-lock bags with a red band above the snap-lock closure measuring approximately 95 millimetres (mm) long by 50 mm wide were selected for this study. These ten packets were purchased over a period of seven months from nine different commercial outlets in Singapore (Outlets A to I). Two packets were purchased from the same outlet (i.e. Outlet B). The number of bags in each packet ranged from 20 to 104. A total of 855 bags were examined.

Methods

All bags from the ten packets were first examined under a polarised light table, followed by marks examination using the comparison microscope and finally, the measurements of the bags were taken.

Polarising Patterns:

The 855 bags were examined under a polarised light table for the purposes of:

a. Screening and classification of the bags into groups with similar polarising patterns to facilitate manufacturing marks examination.
b. Examination of the variation in polarising pattern within a packet.

Manufacturing Marks:

After classification by polarising patterns, the manufacturing marks of each group of bags were examined using comparison microscopy to determine their manufacturing sequences.

Measurements:

The parameters (weight, dimensions and thickness) of all the bags from each sequence were measured. The weight and dimensions were measured using an analytical balance and a ruler respectively. The locations where the dimensions and thickness measurements were taken are illustrated in Figure 1.

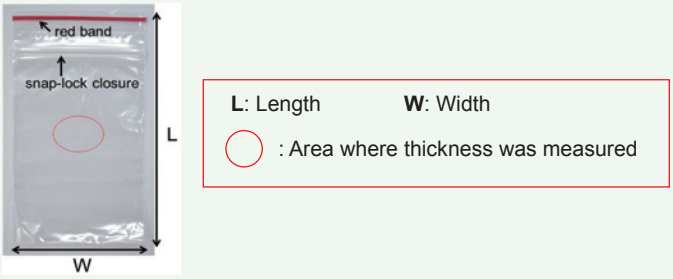

Figure 1: Measurements taken of bags

Results and Discussion

Polarising patterns:

Polarising pattern examination was found to be the most efficient screening method for comparing large quantities of bags. The findings were as follows:

a. Within each of the ten packets of bags, one type of polarising pattern was found. It should be noted that in our studies of other types of snap-lock bags, there were more than one type of polarising pattern in a packet.
b. A total of six types of polarising patterns were identified from the ten packets of bags. Refer to Figure 2 for these six polarising patterns and Table 1 for the outlets with bags having these patterns and the quantity of bags for each pattern.
    i. Packets of bags from different outlets (i.e. from outlet E and F) were found to have similar polarising patterns.
    ii. Conversely, packets of bags from the same outlet can have different polarising patterns, for example, the two packets of bags from outlet B had different polarising patterns.

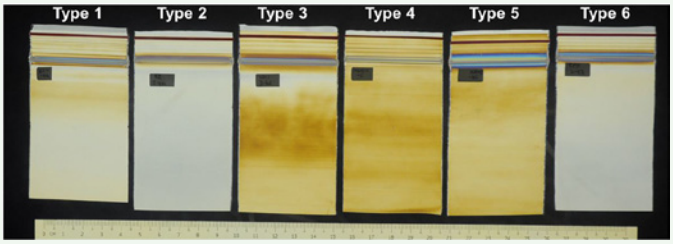

Figure 2: Six types of polarising patterns.

Type	1	2	3	4	5	6
Outlets	A, B (Pack 1), C	B (Pack 2), D	E, F	G	H	I
Number of bags	304	203	124	102	20	102

Table 1: Outlets of purchase and the number of bags for each type of polarising pattern.

Manufacturing Marks

A total of 15 sequences of consecutively manufactured bags consisting of 852 bags were found. Three bags from two packets (Outlets C and F) could not be associated to any of the 15 sequences as well as among themselves to form manufacturing sequences. For these 15 sequences:

a. Each sequence contained bags from the same outlet. Hence, even though some bags from different outlets had similar polarising patterns, the bags from different packets were clearly not associated to each other from the examination of manufacturing marks.
b. Each packet of bags had between one to four manufacturing sequences. The presence of more than one sequence in a packet could be due to the removal of some bags as part of quality assurance checks or due to the random packing process by the manufacturer or repacking by the retailer.
c. The number of bags in each sequence ranged from two to 102.

Measurements:

The internal variation (difference between the measured minimum and maximum values) in each parameter for the 15 sequences was determined. The variations in these parameters were compared among the 15 sequences. The minimum, typical (most frequently encountered variations) and maximum variations in each parameter are tabulated in Table 2. The number of sequences and the number of bags per sequence having the minimum, typical and maximum values are also included in Table 2. Using the length parameter as an example, 2 out of the 15 sequences had a minimum variation of 0.5 mm in length. The typical variation in length was between 1.0 mm to 2.0 mm and this was exhibited by 8 of the 15 sequences. The maximum variation in length was 4.0 mm and only 1 of the 15 sequences exhibited this variation. The remaining 4 sequences had variations between 0.5 mm to 4.0 mm in length.

There was no direct association between the internal variation in each parameter and the number of bags in each manufacturing sequence. For example, the minimum internal variation in the length of the bags was found to be 0.5 mm. The number of consecutively manufactured bags having this minimum value can be as few as a 10-bags sequence to as many as an 85-bags sequence. Similarly, for the typical variation in length value, sequences with as few bags as 10 bags per sequence or as many bags as 102 bags in sequence can show the same variation.

The absence of association between the internal variation in each parameter and the number of bags in each manufacturing sequence indicated the importance of quality control in the production of plastic bags as the length, width and thickness are typically pre-defined parameters in a production. Small variations even across many bags (hundred or more) suggest a well-controlled production. Conversely, large variations across a small number of bags suggest a manufacturing process that is less controlled.

The maximum internal variation (highlighted in yellow) in each parameter found in this study will be used as a reference in casework for the association and discrimination of bags in the range of this size (~95.0 mm by ~50.0 mm). In case examination, the manufacturing quality of the bags should be taken into consideration too as the quality will affect the variation of the physical parameters.

Range of length of 855 bags: 87.0 mm to 95.0 mm

Parameter	Internal variation		
	Minimum	Typical	Maximum
L (mm)	0.5	1.0 to 2.0	4.0
No. of sequences	2	8	1
No. of bags per sequence	10 to 85	10 to 102	97

Range of width of 855 bags: 49.0 mm to 53.5 mm

Parameter	Internal variation		
	Minimum	Typical	Maximum
W (mm)	0.5	1.0	3.5
No. of sequences	3	7	1
No. of bags per sequence	2 to 10	3 to 102	100

Range of thickness of 855 bags: 0.065 mm to 0.110 mm

Parameter	Internal variation		
	Minimum	Typical	Maximum
Thickness (mm)	0.005	0.010	0.030
No. of sequences	2	6	1
No. of bags per sequence	2 to 4	3 to 102	102

Range of weight of 855 bags: 449.9 mg to 572.8 mg

Parameter	Internal variation		
	Minimum	Typical	Maximum
Weight (mg)	2.7	35.0 to 49.0	78.2
No. of sequences	1	5	1
No. of bags per sequence	2	20 to 102	97

Table 2: Range of internal variation, number of sequences and number of bags per sequence.

Conclusion

The minimum, typical and maximum internal variations in the physical parameters as well as the variation in polarising patterns of the 15 sequences of consecutively manufactured bags provided a range of acceptable internal variations to use as a reference against the discrimination of plastic bags commonly encountered in casework. Moving forward, we will continue to examine the physical characteristics of more bags from these nine outlets to determine if there are changes in these characteristics over time. We will also examine bags from different outlets to determine similarities and differences in physical characteristics between different outlets.

Transferability and Persistence of Paint Evidence in Car Vandalism Incidents

Ms. Li Yihua, Ms. Leong Wai Ying, Mr. Lim Thiam Bon, Ms. Lim Chin Chin
Health Sciences Authority, Singapore

Introduction

Paint evidence is often collected when there is a transfer between two contacting surfaces. These evidences that typically occur in trace levels can be used to provide association and establish links between the suspect and known sources. The application of paint evidence to provide such links is common for cases involving traffic accidents and break-ins, but less for vandalism incidents involving scratching of vehicles. The occurrence of paint evidence on tools used for such vandalism incidents is relatively uncommon as paint scratched off a vehicle usually occurs as loose fragments that do not persist for long on the tool. The first case study in this paper illustrates the possibility of using paint evidence to associate the suspect involved in serial car-vandalism incidents. The second case study demonstrates that paint evidence does not persist on tools for long, but that the repository where the tool was found had more significance. The importance of proper collection of samples is also discussed.

Case Study 1

A bunch of four keys and a pair of foldable scissors were seized from a suspect implicated in several car-vandalism incidents. Upon microscopic examination, two small gold-coloured vehicle paint fragments were found in the debossed markings on the bows of two keys. Both fragments consisted of three paint layers and were about 0.6 mm by 0.4 mm in size. Investigators were informed of the findings and the involved champagne-coloured car was brought in for comparison samples to be collected. Comparison samples scraped from the scratched bonnet were found to be indistinguishable from the two gold-coloured fragments recovered from the keys. Figure 1 shows the photographs of one of the two keys and the vehicle paint fragment found on it.

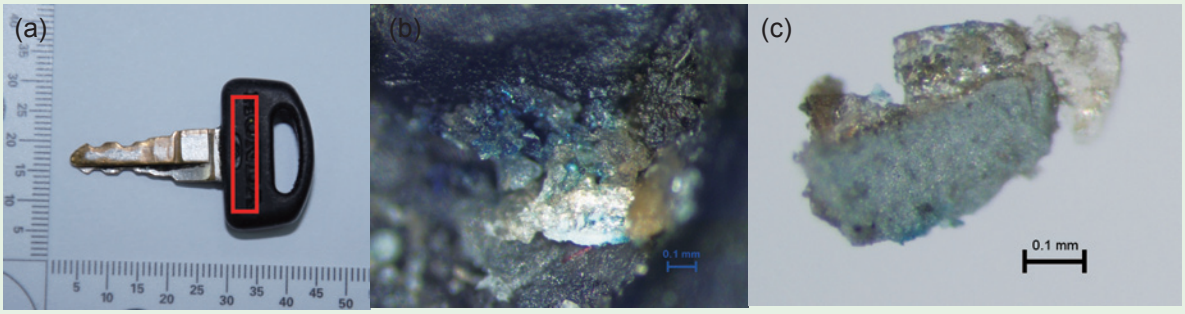

Figure 1: (a) A key with vehicle paint found in the indicated region [red box]. (b) Photomicrograph of vehicle paint fragment in the debossed marking [red box in (a)]. (c) Photomicrograph of a vehicle paint fragment.

Case Study 2

A suspect tool which was improvised using a screw attached to a pen cap and a pair of shorts worn by a suspect were recovered and examined. Many loose materials and a few multi-layered fragments were found on the suspect tool. These materials and fragments were no larger than 0.2 mm in size. In comparison, more than 90 multi-layered fragments were found in the pockets of the pair of shorts worn by the suspect. These fragments were mostly between 0.4 mm and 0.6 mm in size. Through laboratory examination, it was found that 20 of about 50 fragments examined could be associated to 7 of 12 cars examined. The remaining 30 fragments could be from other cars involved but not brought in for comparison. Figure 2 shows photographs of the loose fragments recovered from the tool and multi-layered fragments recovered from the pair of shorts.

This case study illustrates that tools generally do not retain transferred fragments and materials well, particularly the larger fragments. However, the repository where the tool could possibly be kept, such as the shorts in this case, may be of significant evidential value and should be recovered for examination.

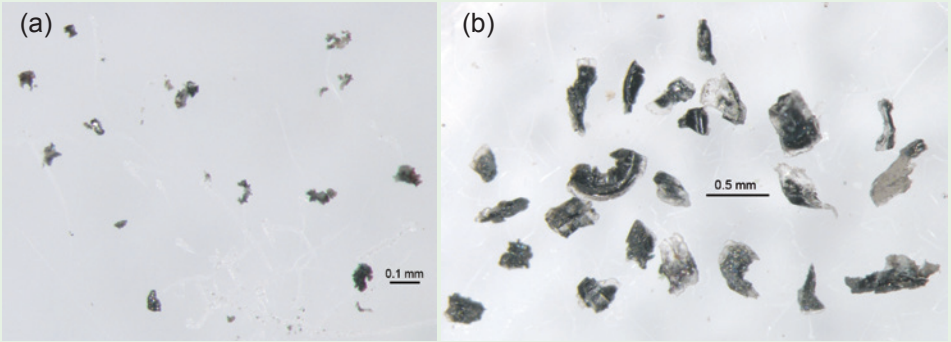

Figure 2: (a) Photomicrograph of loose materials recovered from the suspect tool. (b) Photomicrograph of multi-layered fragments found in the pockets of the suspect's pair of shorts.

Collection of paint samples

Comparison paint samples are typically collected near damaged areas. For vandalised cars, the comparison paint samples should be collected from vehicle parts that were scratched. To minimise the chances of false negative associations for cars with scratches on different parts, comparison samples from each part should be collected as they may have different paintwork. In Case Study 2, many of the cars were found to have refinished paint layers instead of the original equipment manufacturer (OEM) paint layers. Out of 8 cars from which samples were collected from both the left and right doors, 6 of them were found to have different paintwork on both doors. The laboratory has also encountered another case of car vandalism in which the right front and right rear door were found to be indistinguishable in terms of appearance and layer sequence, but differed in the chemical composition of the outermost clearcoat. Therefore, known samples should also be collected from doors on the same side of the car which shows damage.

Improper collection of samples may also affect the outcome of such cases. In another two cases encountered by the laboratory, the samples were not properly collected; cotton swabs were used to swab the hands of the suspects or the tools used by the suspects, and the scratched parts of the cars as the comparison samples. Vehicle paint materials were not found from the swabs of the suspects' hands and the tools. As for the three swabs obtained from the cars, a few outermost clear-coat materials were found for one swab, a few small two-layered fragments were found for another swab, and no vehicle paint material was found for the last swab. Although swabs may retain paint fragments, these fragments are small, difficult to recover, and may not have all the relevant layers. Recovery of paint materials using cotton swabs is unlikely to yield transfer evidence that provides good evidential value, if any at all. Known samples from cars for comparison should always be scraped from the car.

Conclusion

1. Paint fragments from the damaged part of a vehicle are likely to be transferred to the tool which was used to scratch it. As paint evidence generally does not persist for long on the tool, what does remain on the tool tends to be very small and few in number. Hence, proper preservation of such evidence prior to submission to the forensic laboratory is critical. In Case Study 1, comparison of fragments recovered from a bunch of keys could successfully associate these keys as tools used to scratch the cars.
2. Other than the tool, the repository where the tool was found should be subjected to laboratory examination as it is likely to yield better findings. In Case Study 2, many more paint fragments which were larger in size and had more layers for comparison were found in the pockets of the pair of pants worn by the suspect, compared to the paint evidence recovered from the tool.
3. When scratches are present on different parts of a car, known samples should be collected from these parts separately, e.g. doors on opposite sides should be assumed to have different paintwork.
4. Cotton swabs should not be used to collect paint fragments.

1.	National Forensic DNA Profiling Laboratory, Bangladesh	19.	Guangzhou Forensic Science Institute, People's Republic of China
2.	Department of Scientific Services, Brunei Darussalam	20.	Institute of Forensic Science, Ministry of Public Security, People's Republic of China
3.	Centre for DNA Fingerprinting and Diagnostics (CDFD), India	21.	Institute of Forensic Science, Tianjin Public Security Bureau, People's Republic of China
4.	Department of Police Medicine of the Indonesian National Police, Indonesia	22.	The Institute of Evidence Law and Forensic Science, China University of Political Science and Law, People's Republic of China
5.	Eijkman Institute for Molecule Biology, Indonesia	23.	Laboratory Service, Philippines Drug Enforcement Agency, Philippines
6.	Forensic Laboratory Centre of Indonesian National Police Headquarters, Indonesia	24.	National Bureau of Investigation, Philippines
7.	Indonesian Association of Forensic Pathologist, Indonesia	25.	Natural Sciences Research Institute, University of the Philippines Diliman Quezon City, Philippines
8.	Laboratory of National Narcotics Board, Indonesia	26.	Health Sciences Authority, Singapore
9.	National Digital Forensic Centre (NDFC) of Supreme Prosecutor's Office, Korea	27.	Central Institute of Forensic Science, Thailand
10.	National Forensic Service, Korea	28.	Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University, Thailand
11.	Scientific Investigation Laboratory, Korea	29.	Department of Forensic Medicine, Faculty of Medicine, Siriraj Hospital, Thailand
12.	Food and Drug Quality Control Center, Lao PDR	30.	Department of Medical Sciences, Thailand
13.	Forensic Science Department of Judiciary Police, Macau SAR	31.	Faculty of Medicine, Chiang Mai University, Thailand
14.	Department of Chemistry, Malaysia	32.	Human Genetics Unit, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Thailand
15.	Royal Malaysia Police Forensic Laboratory (RMP Forensic Lab), Malaysia	33.	Institute of Forensic Medicine, Police General Hospital, The Royal Thai Police, Thailand
16.	Mongolian National Institute of Forensic Science, Mongolia	34.	Office of Narcotics Control Board, Thailand
17.	Forensic Science Division, Department of Fujian Provincial Public Security, People's Republic of China	35.	Vietnam Forensic Science Institute, Vietnam
18.	Forensic Science Center of Guangdong Provincial Public Security Department, People's Republic of China		

(As at 26 September 2013)

Upcoming Events

Date	Venue
17 Feb - 22 Feb 2014	American Academy of Forensic Sciences (AAFS) 66th Annual Scientific Meeting. Seattle, Washington, USA.
10 Aug - 16 Aug 2014	International Association for Identification (IAI) Annual International Educational Conference. Minneapolis, Minnesota, USA.
11 Aug - 15 Aug 2014	American Society of Questioned Document Examiners (ASQDE) 72nd Annual General Meeting. Honolulu, Hawaii, USA.
29 Sep - 2 Oct 2014	25th International Symposium on Human Identification (ISHI). Phoenix, Arizona, USA.
13 Oct - 18 Oct 2014	World Forensic Festival, Seoul, Korea International Association of Forensic Sciences (IAFS) 20th World Meeting. AFSN 6th Annual Meeting and Symposium. Asia Pacific Medico-Legal Association (APMLA) 5th Meeting.
19 Oct - 24 Oct 2014	Society of Forensic Toxicologists (SOFT) Annual Meeting, Grand Rapids, Michigan, USA
9 Nov - 13 Nov 2014	The International Association of Forensic Toxicologists (TIAFT) 51st Annual Meeting. Buenos Aires, Argentina

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