ForensicAsia

THE ASIAN FORENSIC SCIENCES NETWORK NEWSLETTER | ISSUE 6 | 2014

AFSN President's Address

Dear Colleagues,

In October 2014, we came together in Seoul, Korea for the 6th Annual AFSN meeting. A year has passed since we met in Singapore for the 5th Annual AFSN meeting where we received a warm welcome by the organising committee last November. Thanks to the efforts of the host institute, Health Sciences Authority, Singapore, and former president Mr. Lim Kong Boon, it has become a lasting memory. The 6th AFSN meeting was held with other valuable meetings, IAFS 2014 (20th World Meeting of the International Association of Forensic Sciences), APMLA 2014 (5th Meeting of Asia-Pacific Medico-Legal Agencies) and WPMO 2014 (10th World Police Medical Officers) under the name of WFF 2014 (World Forensic Festival). Furthermore, AAFS (American Academy of Forensic Sciences), ANZFSS (The Australian and New Zealand Forensic Science Society), ENFSI (The European Network of Forensic Science Institutes), IFSA (The International Forensic Strategic Alliance) and IOFOS (International Organization for Forensic Odonto-Stomatology) joined together as partner associations. With the participation of various associations in the meetings, there were more opportunities for us to broaden our horizons by strengthening the relationship with them than at any other previous meetings. Therefore many of us were able to take advantage of this meeting and bring home close connections.

Since this June, Dr. Angeline Yap has taken over the position of International Liaison Officer and the presidency of IFSA, as Dr. Lam Kian Ming has resigned from HSA. On behalf of the AFSN members, I would like to give him a big round of applause for his devotion and effort for laying the foundations of AFSN as well as its development. There is no doubt that Dr. Angeline Yap is perfectly competent as the new ILO and IFSA president from her positive attitude and contributions to AFSN matters.

Last but not least, I would like to express my appreciation to the AFSN board members, Workgroup chairs and vice chairs for their generous support and continuous attention.

As the president of AFSN as well as the president of WFF 2014, it was a pleasure meeting many of you in Seoul in October.

Dr. Joong-Seok SEO
AFSN President
WFF 2014 President
Director General of National Forensic Service, Korea

AFSN News

Collaborative DNA Testing Exercise for AFSN Member Institutes

The DNA Profiling Laboratory of the Health Sciences Authority, Singapore will be organising a collaborative DNA testing exercise for the member institutes of the AFSN. The objectives in piloting this exercise are:

– to collect and exchange information among the member institutes;
– to provide insight to the level of competence within the region; and
– to enhance cooperation among member institutes to meet quality standards.

The testing exercise will commence in early 2015. Sample packs consisting of two known stains and two questioned stains will be provided to participating member institutes. Results from STR analyses are to be submitted before the due date. After data collation and evaluation, a summary report will be made available to participating member institutes in mid-2015.

Eight member institutes had expressed interest during the 6th AFSN meeting in Seoul, Korea. Thank you for your support. More information will be sent to you very shortly.

If there are any queries, please contact Mr Tan Wei Jie at Tan_Wei_Jie@hsa.gov.sg.

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Editor's Address

Dear members and friends of AFSN,

As the year 2014 draws to a close, one of the biggest highlights was the World Forensic Festival 2014 which was hosted by the National Forensic Service, Korea. This international event brought together several important meetings – 6th AFSN Meeting, 20th International Association of Forensic Sciences Meeting, 5th Meeting of the Asia-Pacific Medico-Legal Agencies, and the 10th World Police Medical Officers Meeting. I would like to take this opportunity to congratulate and thank the National Forensic Service, Korea, for a wonderful and thrilling Festival.

This issue of Forensic Asia highlights some changes to the editorial committee. Dr Lam Kian Ming has left Health Sciences Authority. We would like to express our appreciation to Dr. Lam Kian Ming who has been an invaluable AFSN Board member and Chief Advisor to the Forensic Asia editorial committee. We wish him all the best in his future endeavours. We would also like to extend our congratulations to Dr. Angeline Yap, our Editorial Advisor, who is now the new AFSN International Liaison Officer and President of IFSA.

In this issue, we have a compilation of 13 articles. There is also a proposal of a collaborative DNA testing exercise for the AFSN member institutes. Participation in collaborative testing exercises is a routine amongst the member institutes in the European Network of Forensic Science Institutes (ENFSI) and serves invaluable roles in inter-laboratory learning, enhancement of methods, and assessment of new technological platforms. I believe this exciting project can strengthen cooperation and collaboration amongst our member institutes to new heights, and hope that such a project can extend beyond DNA to the other forensic disciplines.

I would also like to take this opportunity to express my thanks and gratitude to all the editorial committee members for their support and assistance rendered in reviewing the articles in this issue. We hope that all of you will enjoy reading the interesting articles and we look forward to seeing more submissions for the next issue! Please do give us your feedback and comments at hsa_asg@hsa.gov.sg.

Assoc Prof Christopher Syn
Editor

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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.

38th ENFSI DNA Working Group Meeting

Ms. Wong Hang Yee, Ms. Lim Xin Li
Health Sciences Authority, Singapore

The European Network of Forensic Science Institutes (ENFSI) DNA Working Group Meeting is an annual scientific meeting that gathers forensic DNA professionals from all over Europe to share and present current information, research and training opportunities in this rapidly expanding field. It also serves as a platform for the formulation and promulgation of ENFSI DNA analysis and quality assurance guidelines. This year, the 38th ENFSI DNA Working Group Meeting was hosted by the National Forensics Bureau, Tbilisi, Georgia from 22 to 25 April. The Biology Division, Health Sciences Authority, Singapore, as a guest member of the European DNA Profiling Group (EDNAP), was invited to attend the ENFSI DNA Working Group Meeting.

The first day of the meeting was dedicated to the EDNAP meeting and the ENFSI DNA Working Group CODIS Users meeting. In the following three days, there were five different workgroups that addressed different areas of interest in the forensic community. The five workgroups were:

1. QC, QA, Sampling kits, Training and Teaching
2. DNA Analysis Methods and Interpretation
3. Establishing a DNA Database – Legislative and Laboratory Issues
4. Automation and LIMS subgroup – Automation and Expert Systems
5. Forensic Biology

In the EDNAP and CODIS users meetings, the number of participants in each session was kept small to allow for active discussion and decision-making among the member institutes. Updates on joint exercises that member institutes had participated in, such as the mRNA, IrisPlex and ancestry markers, were presented in the EDNAP Meeting. Besides, news from EDNAP forensic mtDNA population database (EMPOP), Interpol, EUROFORGEN-NoE and challenges of interpreting the relevance of trace DNA were also reported during the session. In the CODIS Users Meeting, updates were given by the FBI. Representatives from each country also shared their experiences with CODIS and the challenges that they faced. This allowed for active exchange of useful information between the FBI and users.

During the break-out into the five workgroups, there were open discussions and participants were willing to share their experiences in their institutions. This helped to facilitate the exchange of information and ideas between one another. At the end of the workgroup meetings, a summary was presented by the Chair of the different workgroups to all attendees. Dr. Christopher Syn, Director of the Biology Division, HSA, presented on the formation and history of AFSN, the network within the Asian region equivalent to the European ENFSI, as well as an introduction to the forensic laboratories in HSA.

Besides the informative sessions, the host institute also thoughtfully arranged activities for participants to immerse in the rich culture and history of Georgia. The authentic Georgian cuisine and the visit to the magnificent Svetitskhoveli Cathedral built in the 4th century in the ancient city, Mtskheta, were memories that would be fondly remembered.

Activities of the International Forensic Strategic Alliance (IFSA) in 2013

The Annual IFSA Meeting at Lyon, France on 6-7 October 2013

Representatives of the six regional networks (AFSN, AICEF, ASCLD, ENFSI, SARFS and SMANZFL) and IFSA strategic partners (UNODC and INTERPOL) met on 6 and 7 October 2013 at INTERPOL General Secretariat Headquarters, Lyon, France.

One of the important items for discussion at the meeting was the creation of the IFSA Minimum Requirement Documents, where the objectives of this initiative as well as the workflow for the process were thoroughly deliberated. IFSA Board upheld the importance of the Minimum Requirement Documents as a means of assisting emerging forensic laboratories in developing countries to establish a baseline or starting point that must be followed in order to achieve reliable results. Forensic providers are encouraged to build on this foundation and strive to continually improve the quality of services provided.

The document addresses the following framework:

1. Competence of Personnel
2. Equipment and Consumables
3. Collection; Analysis; Interpretation; Reporting
4. Procedures; Protocols; Validation
5. Quality Management

Members of IFSA at the IFSA Annual Meeting, Lyon, France

IFSA Website

Following the annual meeting in Lyon, the IFSA website hosted at http://www.enfsi.eu/ifsa was also revamped and now carries more information such as the vision, goals and function, board members, history of IFSA, Minimum Requirement Documents, annual board meetings, presentations and documents. In particular, the procedure for the approval of the Minimum Requirement Documents and some FAQs associated with these documents were also available on the website.

Group photograph of the 17th International Forensic Science Managers Symposium

Participation at the 17th INTERPOL International Forensic Science Managers Symposium, Lyon, France 8-10 October 2013

The IFSA Board Members participated in the 17th INTERPOL International Forensic Science Managers Symposium, Lyon, France 8-10 October 2013 and presented the following two posters at the symposium:

1. Introduction to IFSA
2. IFSA Minimum Requirement Documents

IFSA launched Minimum Requirement Documents at WFF2014

IFSA launched the first series of three Minimum Requirement Documents (MRD) at the World Forensic Festival (WFF) 2014 which was held from 12 to 18 October 2014 in Seoul, Korea. The three documents are in the forensic areas of crime scene investigation, DNA analysis, and identification of seized drugs.

IFSA would like to thank the many scientific working groups and experts from the six regional forensic science networks, as well as many individuals and IFSA strategic partners who have made invaluable contributions during the various rounds of consultations and discussion.

IFSA members at the launch of MRD at the WFF2014

The International Criminal Court

Prof. Tony Raymond
Chief Scientist, Strategic Forensic Support Branch, NSW Police Force, Australia
International Forensic Strategic Alliance representative to the International Criminal Court

The International Criminal Court (ICC), governed by the Rome Statute, is the first permanent, treaty-based international criminal court established to help end impunity for the perpetrators of the most serious crimes of concern to the international community. These typically include war crimes, crimes against humanity, genocide, rape, use of child soldiers, etc.

The ICC is an independent international organisation, and is not part of the United Nations system. Its seat is at The Hague in the Netherlands. Although the Court's expenses are funded primarily by States Parties, it also receives voluntary contributions from governments, international organisations, individuals, corporations and other entities.

The international community has long aspired to the creation of a permanent international court, and, in 1998, reached a historic milestone when the Rome Statute, the legal basis for establishing the permanent ICC, was adopted. The Rome Statute entered into force on 1 July 2002. There are now 122 countries who are parties to the Rome Statute.

The Office of the Prosecutor (OTP) is one of the four organs of the Court.

The Office investigates and prosecutes genocide, crimes against humanity and war crimes committed by nationals of a State Party or on the territory of a State Party on or after 1 July 2002.

The OTP is currently investigating eight situations:

1. Uganda
2. Democratic Republic of Congo
3. Darfur, Sudan
4. Central African Republic
5. Kenya
6. Libya
7. Côte d'Ivoire
8. Mali

The OTP is also currently conducting preliminary examinations analysing alleged crimes committed on the territories of Honduras, Afghanistan, Korea, Central African Republic, Comoros, Ukraine and Iraq, and assessing if there are genuine national proceedings being carried out in Georgia, Guinea, Colombia and Nigeria.

This OTP had limited internal scientific support and determined that it needed a Scientific Advisory Board in support of its investigative and prosecutorial mission. To that end, chairpersons and presidents or their respective delegates of global forensic networks were invited to The Hague by the ICC. On 25 June 2014, at the conclusion of the inaugural meeting of this group, the OTP formally established the Scientific Advisory Board (SAB).

The SAB reviewed and certified the Standard Operating Procedures of the OTP relating to, inter alia, human remains recovery, autopsies, forensic clinical examinations and crime scene examinations at the inaugural meeting.

The SAB will provide recommendations to the Prosecutor on the most recent developments in new and emerging technologies, scientific methods and procedures that can reinforce the capabilities of the Office in the collection, management and analysis of scientific evidence relating to the investigation and prosecution of crimes of genocide, crimes against humanity and war crimes.

The OTP often conducts its investigations in the midst of on-going volatile situations or post-conflict environments, with shorter windows of opportunity to collect, store and preserve evidence, due to security or other challenges. "The work of the SAB will be crucial to the OTP's efforts, as reflected in its new Strategic Plan, to strengthen its investigative capabilities and enhance the quality of its deliverables when it comes to scientific evidence collection and analysis".

In the words of the OTP Prosecutor Mrs. Fatou Bensouda, "The potential for innovative input and contribution of the Board is limitless on the strength of the wealth of knowledge and expertise of its members, but also due to the richness of diversity and geographical representation of its composition". The Prosecutor further stated that "the law and scientific innovation is not a marriage of convenience, but one of necessity, and together, they make a powerful couple indeed."

The (currently) 16-member board, which includes the IFSA Chair nominee - delegate Prof. Tony Raymond, will meet once a year, and will be presided over by its newly elected Chair, Prof. Dr. Duarte Nuno Vieira of Portugal.

The Prosecutor Mrs. Fatou Bensouda (front row, second from left) together with the attendees at the inaugural Scientific Advisory Board Meeting.

Forensic Handwriting Discipline of Laboratory of Scientific Police of Judicial Police

A Preliminary Study - A Statistical Workload Analysis

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[2] European Network of Forensic Handwriting Experts (ENFHEX)
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[6] European Academy of Forensic Science (EAFS)

Abstract

This study has two aims. The first aim is to analyse data of recent years from the Laboratory of Scientific Police of Judicial Police (LPC/PJ – *Portuguese abbreviation*) in Portugal and from the Forensic Handwriting (FH) discipline in particular. The second aim is to evaluate the *status quo* and overall results of the Forensic Handwriting discipline in this Laboratory in terms of the workload and approach to requested examinations. 350 examinations from the year 2013 were randomly selected containing 840 questioned documents. In this survey, we inferred that 50% of these documents were related to banks. Furthermore, examinations that included just signatures or signatures with texts represented 92% of forensic analysis and in the 606 results observed, 54.8% were conclusive probabilities.

Introduction

The main goal of the Laboratory of Scientific Police of Judicial Police (LPC/PJ) in Portugal is to provide forensic examinations resulting from criminal offences under the investigation of the Judicial Police (PJ), prosecutors and other official entities. The LPC/PJ has scientific and technical autonomy to search, collect, process evidence, issue expert reports in several domains of forensic sciences and provide witness testimonies in courts of law.

The LPC/PJ is a National Unit of the PJ which was formed in 1957 [1] but only started to operate fully in 1960. The Laboratory originally consisted of five forensic disciplines, namely Toxicology, Biology, Physic-Chemical, Documents and Ballistic. At the beginning, experts were sent to the Germany Bundeskriminalamt (BKA) to undergo professional internship.

From 2009 to the present, the Laboratory has three major departments: *Biotoxicology*, which includes the forensic disciplines of Biology, Toxicology and Chemistry; *Physical-Documental*, which refers to Document, Handwriting, Banknotes, Ballistic and Physical examinations; and *Criminalistic*, which includes Fingerprint and Crime Scene. The Laboratory is currently in the process of adding Forensic Speech and audio analysis to the existing disciplines.

Due to the importance of material evidence in criminal investigations, the number of examinations requested for all forensic disciplines has increased since its beginning and its first examination was performed in the Forensic Handwriting discipline. In the year 1970, this Laboratory conducted 351 examinations. By 1980, the number of examinations increased almost ten-fold to 3,042. A decade later, the number of examinations doubled to 6,694 and in year 2000 it exceeded twenty thousands. In year 2013, the number of requested examinations were 28,545 and the number of concluded ones were 30,800 [2]. In early 2010, 8,863 examinations were pending but in early 2014, the backlog was reduced to 1,719 examinations.

The LPC/PJ has 217 employees scattered across its headquarters and Directories/Departments of Criminal Investigation. The FH discipline has 17 specialists analysing authenticity of signatures and handwriting authorship using comparisons with specimen signatures and handwritings [3]. Proper equipment is used to detect handwriting indentations as well as identifying methods involved in forgery of signatures or text productions.

Table 1 illustrates the number of requested FH examinations in the last four years as well as the response ability of forensic specialists to those requests. As we can see in the table below, in the last three years, this discipline had concluded and sent to court more reports than those that were requested, which means that the backlog of FH has decreased. By the end of July this year, the backlog is less than 200 examination requests. At present and according to the performance of the experts of this forensic discipline, more than 200 requests can possibly be concluded each month, which means that the backlog is cleared, and that FH has a response time of one month. Ultimately, this recovery will facilitate faster administration of justice in court cases involving handwriting forgeries.

Year	Requested examinations	Concluded examinations
2011	2352	1820
2012	2008	2800
2013	1910	2887
2014 (1st quarter)	685	826

Table 1: Number of FH examinations requested and concluded between the years 2011 and 2014 (1st quarter).

In 2012, backlog reduction was observed, due to the following factors:

- Formation of working teams with less experts each, compared to previous years, where there was only one big working group with a diffused task distribution;
- Efficient distribution of examinations;
- Specimen handwritings were collected by the requesting entities, by following instructions and filling in forms available on the PJ website;
- Enhanced human resources management with a clearer task distribution;
- Revised procedures and simplified methodologies;
- Reducing bureaucracy and improvements in administrative flow.

Ultimately, the commitment of FH staff in their work is of utmost importance. Without them, changes in the workflow and backlog reduction would have never been possible.

Materials and Methods

For this statistical analysis, 350 cases from the year 2013 were randomly selected comprising 840 questioned documents, and 581 specimen handwritings of suspected individuals were collected for comparison.

Each case was analysed using different parameters:

- Types and amount of documents;
- Types of analysis / examinations;
- Conclusions.

All data was recorded and processed in a *Microsoft® Excel 2007 file*.

Results and Discussion

A wide diversity of documents had been sent for analysis but the ones related to banks consisted of more than 50% (Figure 1).

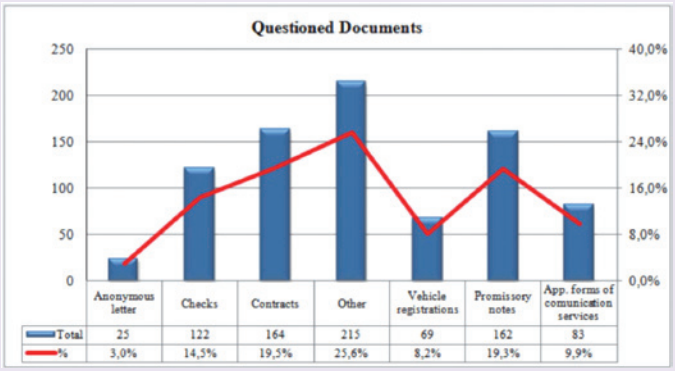


Figure 1: Type of questioned documents examined in 350 Forensic Handwriting cases.

The documents referring to vehicle registrations and application forms of communication services also exhibited significant values since each one of these categories represented about 10%.

Lengthy and laborious examinations are usually necessary in determining the authorship of anonymous letters which corresponds to 3% of all analysed documents.

Another relevant parameter was the requested types of analyses, namely, signatures, text, or signatures and text.

A signature in a document represents a personal or professional commitment. Therefore, forging a signature or denying authenticity of handwritten documents is often tempting. Hence, signature analyses formed the majority of requested examinations to the FH discipline. In fact, signatures plus signatures and text represented 92% of forensic analyses / examinations (Figure 2).

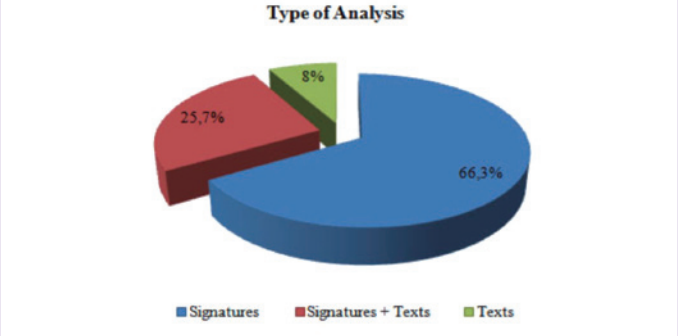


Figure 2: Types of analyses observed in 350 Forensic Handwriting examinations.

In the survey on the 350 cases, we observed 606 results with 54.8% of conclusive probabilities. For authenticity and authorship determinations, positive probabilities were 24.8% and negative probabilities were 30% (Figure 3).

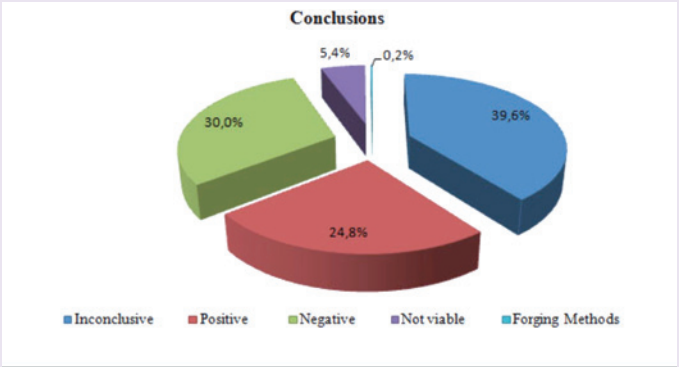


Figure 3: Classification of the conclusions derived in 350 Forensic Handwriting cases.

Conclusions

This study evaluated the *status quo* and overall results of the Forensic Handwriting discipline in the LPC/PJ in terms of the workload, the approach to requested examinations and pendency reduction over the years. The majority of questioned documents are related to banks and most forensic analyses/examinations included signatures or signatures and texts of which about half gave conclusive probabilities.

In future, the Laboratory will carry out a similar study with a larger sample size with extended scope of parameters to be evaluated to have a better understanding of the challenges that the Forensic Handwriting Specialists face in the LPC/PJ. For example, such a study should consider the area of the country from which the requests came from to know the geographical distribution of the requests. Another parameter to examine would be the inconclusive results, cases in which they occur, and their underlying causes.

Also, it would be interesting to study the impact of the results/conclusion from a handwriting examination on the judge's decision.

References

1. Decree-Law nº 41306/57 of October 2. *Diário do Governo Nº 223/57 – I Série*. Ministry of Justice. Lisbon.
2. Farinha, CAL. *Relatório anual de atividade do LPC de 2013*. Judicial Police; 2013.
3. Huber RA, Headrick AM. *Handwriting Identification: Facts and Fundamentals*. CRC Press LLC; 1999.

Digital Forensics Department of CyberSecurity Malaysia

Mr. Mohd Zabri Adil B Talib
Digital Forensics Department, CyberSecurity Malaysia

Our Background

CyberSecurity Malaysia (CSM) is a Malaysian national cyber security specialist agency under the purview of the Ministry of Science, Technology and Innovation (MOSTI). One of the services offered by CSM is digital forensics. CSM has started providing services on digital forensics since 2002. To date, more than 3,000 cases related to digital evidence have been submitted to CSM for analysis.

Our Roles

The Digital Forensics Department of CSM is the Malaysian national reference center for digital forensics. Its main role is to provide law enforcement agencies (LEA) with trusted and reliable services on digital forensics, as well as to assist them in preserving digital evidence at crime scenes. The unique setup of the department, whereby it is not governed under any Acts or any LEA, ensures the impartiality and the trustworthiness of the forensic results.

The Organisation

The department comprises of several units as illustrated in Chart 1: The Organisation.

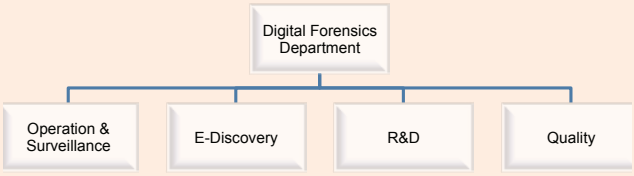


Chart 1: Department Organisation Chart.

The Services

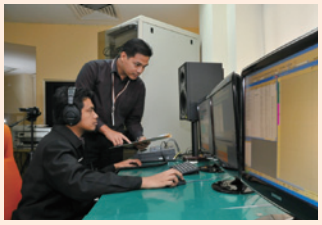
Several services offered by the laboratory to LEA, as well as to private organisations are listed in Table 1: Offered Services.

Services
Computer Forensics
Video Analysis
Audio Analysis
Image Analysis
Mobile Phone Analysis

Table 1: Offered Services.

Computer Forensics Lab

Video Forensics



Audio Forensics

Mobile Phone Forensics

Recognition

Recognition received by the laboratory in the area of digital forensics is as follows:

- Accredited by ASCLD/LAB-International
  The accreditation is in the discipline of Digital & Multimedia Evidence. The lab has been accredited since 2011;

- Analysts are recognized by Malaysia Court of Law as Experts

Analysts of CSM are gazetted under the law of Malaysia, as stated in the Malaysia Criminal Procedure Code 399. This recognises CSM analysts as experts in the Malaysia court of law.

Research Work

Research papers produced by the laboratory are as follows:

1. Algorithm for Tracking Sensitive Information of Online Application in Computer Memory, CSEE, 2014.
2. iOS Anti-Forensics: How Can We Securely Conceal, Delete and Insert Data?, HICSS, 2014.
3. iOS Forensics: How can we recover deleted image files with timestamp in a forensically sound manner?, ARES, 2013.
4. Digital camcorder forensics, AISC, 2013.
5. Data recovery of a DVR hard disk using proprietary file format, IFIP 11.9, 2013.
6. Holistic Approach for Memory Analysis in Windows System, ISICO, 2013.
7. Object Signature Search for Capturing Processes Memory Image of Windows System, International Journal of Computer and Communication Engineering, 2013.
8. Digital Forensics Institute in Malaysia: The way forward, Digital Evidence and Electronic Signature Law Review, 2012.
9. An Analytical Framework for Digital CCTV Forensics and Data Recovery, eForensics, 2012.
10. Investigating the PROCESS block for memory analysis, WSEAS, 2011
11. Hybrid Approach for Memory Analysis in Windows System, International Conference on Electrical, Computer, Electronics and Communication Engineering, 2012.
12. Multiple-frames super-resolution for closed circuit television forensics, IEEExplore, 2011.
13. Forensic Acquisition on MP3 players, IEEExplore, 2011.

For more information, please visit our websites: www.cybersecurity.my and www.cybercsi.my.

National Digital Forensic Center of Supreme Prosecutors' Office, Republic of Korea

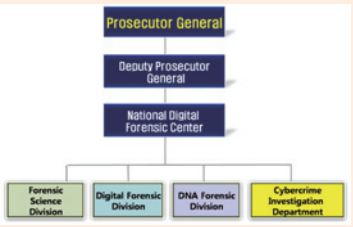
Dr. Seung-Hwan Lee
National Digital Forensic Center, Supreme Prosecutors' Office, Republic of Korea

Introduction

The National Digital Forensic Center (NDFC) is one of the leading organisations in Korea which unravels truth and protects human rights through advanced forensic science. As the incidence of more sophisticated crimes increases, NDFC responds to the changing nature of crimes by assisting investigations with prompt and accurate analysis of evidence. Since the founding of the forensic department at the Supreme Prosecutors' Office (SPO) in 1984, we have made all efforts to establish forensic infrastructure, expand state-of-the-art forensic disciplines, as well as to enhance cooperation with domestic or international institutions.

Organisational Structure

The NDFC is divided into three divisions and one department with more than 120 expert scientists and investigators. NDFC is led by the Forensic Science Planning Office under the control of the Deputy Prosecutor General.



Tasks and Responsibilities

NDFC's missions can be categorised as follows:

1. Evidence examination: The results are submitted to the court for the evaluation of evidential value;
2. Research and development: Introduction and validation of new forensic techniques;
3. Planning and judiciary affairs related to forensic science;
4. Assisting investigation: Collection of evidence from the crime scene.

Forensic Science Division

The Forensic Science Division has established its position in various forensic fields listed below by strengthening authentication and analysis capabilities.

1. Forensic Chemistry
2. Document Examination
3. Psychological Analysis
4. Voice and Image Analysis
5. Fire Investigation
6. Development and Management of Investigation Equipment
7. Planning and Management of Interrogation Video Recording System

DNA Forensic Division

The DNA Forensic Division contributes to the realisation of a crime-free society by providing decisive evidence for the resolution of heinous crimes. Since 2010, we manage a convicted offender DNA database through which numerous serious or serial offenses have been solved. A huge research project is under way to localise and advance cutting-edge DNA technologies. Recently, we have also launched a non-human forensic biology department to combat against illegal foods and drugs.

Digital Forensic Division

As digital media diversifies, the subjects in this field are rapidly expanding. The Digital Forensic Division assists in getting closer to the truth by analysing digital evidence like mobile phones, computers, e-mails and so on. Digital Investigation Network is established within the District Prosecutors' Office to provide prompt support to investigations.

Cybercrime Investigation Department

The Cybercrime Investigation Department protects people from cybercrime by creating a free and safe cyberspace. We perform investigations on major cyber crimes including cyberterrorism, hacking and personal information leakage.

Conclusion

We observe and maintain an international laboratory accreditation system (ISO/IEC 17025) in the fields of DNA, Forensic Chemistry and Document Examination. Our research findings are often published in international forensic science journals, indicating that NDFC is globalised enough to stand shoulder to shoulder with European or American countries. We are next in line to host the 2017 International Society for Forensic Genetics (ISFG) Congress in Seoul. Through such events, and also as a member of AFSN, we will continuously reinforce international collaborative efforts to bridge Asia and other continents.

Harmonising ISO/IEC 27001 and ISO/IEC 17025 Implementation in the Digital Forensics Department, CyberSecurity Malaysia: A Case Study

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Background

Aimed at providing quality and impartial service, the Digital Forensics Department (DFD) of CyberSecurity Malaysia (CSM) decided, in January 2007, to obtain accreditation from an accreditation body, American Society for Crime Lab Director/ Laboratory Accreditation Board (ASCLD/LAB) for its forensics services. The project was scheduled to be delivered in November 2011. The accreditation from ASCLD/LAB was based on the ISO/IEC 17025 *General Requirements for the Competence of Testing and Calibration Laboratories* and ASCLD/LAB's own Supplemental Requirements [1].

In the same year, CSM had successfully been certified with ISO/IEC 27001 Information Security Management System. The scope of the certification covered all departments under the CSM.

ISO/IEC 27001 and ISO/IEC 17025

ISO/IEC 27001 is a standard that provides requirements for an information security management system (ISMS). ISMS is a systematic approach to managing sensitive company information so that it remains secure [2]. It includes people, processes and IT systems by applying a risk management process. It enables small, medium and large businesses in any sector to keep information assets secure. Being certified, CSM assures customers of data confidentiality, integrity and availability.

ISO/IEC 17025, on the other hand, specifies the general requirements for the competence to carry out tests and/ or calibrations, including sampling [3]. It covers testing and calibration performed using standard methods, non-standard methods, and laboratory-developed methods.

Chart 1 shows the requirements or clauses that both ISO standards have defined.

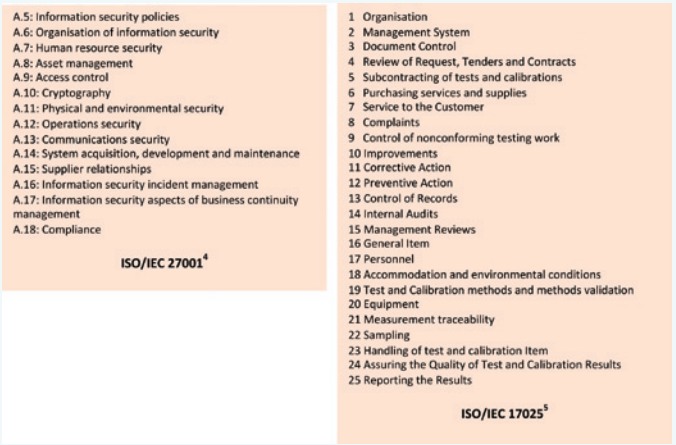

Chart 1: Requirements defined in ISO/IEC 27001 and ISO/IEC 17025.

The Challenge

DFD had to conform to both ISO standards, ISO/IEC 27001 as well as ISO/IEC 17025, if they were to succeed in obtaining the accreditation. This was a challenge to DFD because it seemed like a far-fetched task. The questions that arose at that time were:

1. Can DFD create policies and procedures that are not conflicting with both ISO standards?
2. How many audits does DFD need to go through every year?
3. Will the decision to be accredited in ISO/IEC 17025 affect the current implementation of the ISO/IEC 27001 certification?

In this article, we will share with the readers the methods of harmonising the implementation of both standards.

Harmonising ISO/IEC 27001 and ISO/IEC 17025

During the development of the policies and procedures in January 2008, DFD realised that some of the requirements, for example *Document Control* and *Physical Access Control*, were already in place and implemented by the organisation as a fulfilment to ISO/IEC 27001.

Although ISO/IEC 17025 required DFD to develop the same policies and procedures, the detailed requirements were not the same. For example:

a. ISO/IEC 17025 required management system documents to have end of page marking, whereas ISO/ IEC 27001 did not specify such a requirement.
b. ISO/IEC 17025 required that a list of approved vendors is maintained, whereas ISO/IEC 27001 did not specify such a requirement.

This posed a real challenge to DFD – could DFD harmonise both ISO standards without adversely affecting the whole organisation?

The Mechanism

Several meetings and discussions were conducted in March 2008 between CSM's top management and DFD personnel to solve the matters. Thorough reviews of the requirements of ISO/ IEC 27001 and ISO/IEC 17025 were also conducted to look at similar areas. During the discussions, a level of understanding was reached and the methods for harmonising both standards were developed. Table 1 summarises the understanding and the methods implemented to ensure both standards would work hand in hand.

No.	Matter	Action
1	**No Contradiction between both Standards** Requirements specified in ISO/IEC 17025 and ISO27001 are not in any way contradicting each other. A standard may define more stringent requirements than the other, but never contradict it. With this firm understanding, DFD can then move forward and start to develop its policies and procedures.	An understanding
2	**Supplement, not Supersede** Should DFD need to add more clauses in the already implemented organisation's policies and procedures, DFD may specify the additional clauses in its own policies and procedures. However, reference to the organisation's policies and procedures must be made in the document. For example, CSM has its own Organisation Document Control Procedure, adhered to by all departments. However the clauses in the procedure were not sufficient for ISO/IEC 17025. Thus DFD needs to develop its own Document Control Procedure. This procedure shall not supersede the Organisation Document Control Procedure; instead it will supplement it. To implement this, DFD added the following clause in the opening of its Document Control Procedure: *"Organisation Document Control Procedure shall be adhered at all times. The DFD Document Control Procedure shall define additional clauses to fulfil ISO/IEC 17025 requirements."*	Implementation
3	**Insert a saving clause** Although both ISO standards do not contradict each other, there are some conflicts on the implementation level, between procedures outlined by CSM and those outlined by DFD. For example, according to the Organisation Record Control Procedure, obsolete records shall be disposed in a central obsolete repository. However, since DFD owns classified records that cannot be distributed to internal staff, DFD has to manage its own obsolete records. To implement this, DFD has developed its own Record Control Procedure. A saving clause was then added to the Organisation Record Control Procedure: *"Organisation Record Control Procedure shall be adhered at all times. Some department shall have its own implementation process, in which the department shall define the process in its own Record Control Procedure."*	Implementation
4	**Audit some requirements once, but never twice** DFD shall undergo internal audits twice annually, one for ISO/IEC 27001 certification and the other for ASCLD/LAB accreditation (ISO/IEC 17025). However, auditors for ISO/IEC 27001 certification shall not conduct audits on requirements that have been covered in the ISO/IEC 17025 audit and vice versa. This is done in order to reduce DFD workload.	Implementation

Table 1: An understanding of the standards that has been agreed by team members and the implementation methods of harmonising both.

Summary

The methods that have been put into practice in order to harmonise the implementation of both ISO standards have been successful. DFD has been working with both standards for almost four years without any glitches. The lesson learnt from this implementation is that team members must fully understand the requirements of both standards before making any decision so as to avoid redundant, complicated and lengthy implementation processes.

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Combined Use of 96-Well Centrifugal Filtration Plate with Automation Workstation Improve Efficiency of Touch DNA Detection

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Abstract

We report a design of a 96-well centrifugal filtration plate and its use in an automated DNA extraction method of touched objects. Cells on the touched objects such as cigarette butts, glove prints, swabs from door handles, bottles etc. are lysed in the 96-well filtration plate. The cell lysate is separated from the objects by centrifugation. Automated DNA extraction and purification are then carried out on a robotic workstation. Such a method not only improves DNA profile obtained rate from touched objects but also shortens sample processing time. Processing of 92 samples can be completed in 90 minutes.

Introduction

As DNA technology is increasingly becoming one of the most effective forensic tools to exonerate or convict suspects in criminal cases, there is a growing number of forensic casework samples that require processing. Most of them are touch DNA samples. Automated systems have been increasingly utilised in DNA extraction to minimise human error and to ensure high reproducibility [1]. In our laboratory, for example, we use an automatic liquid workstation, TECAN Freedom EVO150-8 [2,3], to extract DNA from biological samples. Use of such an automatic workstation indeed significantly improves working efficiency and consistency. However, since there is a wide variety of sample types on different carrier materials [4], obtaining high yields and high quality DNA from various sources has proven to be challenging [5].

We have developed a high throughput method of using a 96-well filtration plate (Figure 1) (kindly manufactured by Axygen) to lyse cells on touched objects and then isolating the lysate by centrifugation. The lysate is then subjected to automatic DNA extraction on a liquid handler. In this study, we found that such combined use of 96-well centrifugal filtration plate and an automatic liquid handler not only shortened sample processing time, but also improved DNA profile obtained rate.

Materials and Methods

Sample Collection

Touched objects/swabs were collected from crime scenes, and collection of cells associated with touch biological samples were done with a moist swab followed by a dry swab. Objects included were cigarette butts, beverage bottle, handle, wire connector, tools and glove prints. For the control group (the conventional workstation alone, referring to the automatic liquid workstation, such as TECAN Freedom EVO150-8, provided by the instrument manufacturer), DNA from 536 biological samples were extracted and analysed. For our new method (96-well filtration plate/workstation), DNA from 553 biological samples were extracted and analysed. Use of this special 96-well centrifugal filtration plate requires both a customised heating base (kindly provided by Tecan) and a customised magnetic frame (kindly provided by Axygen).

DNA Extraction and STR Analysis

Touched objects/swabs were placed in the 96-well plate. 800 µl of Lysis buffer (containing Tris-Cl, NaCl, EDTA and SDS) was added into each well, and the plate was spun after incubation in a 99ºC water bath for 10 minutes. The lower part of the plate was then loaded onto a Tecan Freedom EVO 150-8 for automated DNA isolation and purification [6]. The kit used was M48 magnetic beads (provided by Qiagen). The control process was done according to the manufacturer's instructions.

DNA was amplified using Identifiler Plus Kit (Applied Biosystems). GeneAmp 9700 (Applied Biosystems) with Gold block was used for PCR amplification. PCR products were further analysed on a 3500 Genetic Analyzer (Applied Biosystems). The data was analysed with ID-X expert analysis system. Samples showing 13 or more loci were considered to be successful profiling.

Quantitative Analysis

To further evaluate the method, we conducted quantitative analysis in a model system. We added 5 ng, 10 ng and 20 ng of 9947A DNA to each swab. The swabs were processed to extract the 9947A DNA.

The recovered 9947A DNA was amplified and analysed on a 7500 real-time PCR system (Applied Biosystems). SYBR® Green Real-time Master Mix as well as a primer pair specific for human genomic DNA was kindly provided by NuHigh Biotechnologies. Both quantitative analysis and melting curve analysis were done to evaluate DNA recovery and specific amplification. According to the amount of DNA recovered, the recovery rate can be calculated. All measurements were performed in triplicates.

Statistical Analysis

The data were shown as mean ± SD. Comparisons between groups were analysed using the Student's χ^2-test or ANOVA. The significance was analysed with SPSS10.0 software and a p-value < 0.05 was considered to be statistically significant.

Results

Comparison of Efficiency of DNA Detection With or Without 96-Well Filtration Plates

As shown in Table 1, 43.1% of processed samples (231/536) obtained DNA profiles with the conventional method. Among 553 samples processed with our new method, 301 samples yielded DNA profile. In other words, the profile obtained rate is 54.4% which is statistically higher than that of the conventional method (p<0.05).

Profile Obtained Rates of Different Touched Objects

As shown in Table 2 and Figure 2, our newly developed method is significantly (p< 0.05) better than the conventional method with higher profile obtained rate for swabs of door handles (41.1% vs 27.3%), wire connectors (69.1% vs 38.6%) and tools (51.3% vs 33.3%).

Profile obtained rate is also higher with the new method for cigarette butts (97.3% vs 88.9%), glove prints (31.6% vs 19.5%) and swab from bottles (90.9% vs 74.4%). However, there was no significant difference between the two methods.

The New Method Shortens Sample Processing Time Up to 1 hour

With the conventional method, DNA lysate is transferred by liquid workstation. The process is slower than that of the new method in which DNA lysate is transferred by plate centrifugation. With our newly developed method, our process is shortened by up to 60 minutes.

Quantitative Evaluation of DNA Recovery by the Two Methods

We further evaluated DNA recovery yield quantitatively. As we can see from Figure 3A, relative expression of DNA standard using 96-well filtration plates were increased by more than 150%. We also calculated the recovery rate by real-time PCR. Results are shown in Figure 3B. Different amounts of standard 9947A DNA, 5ng, 10ng and 20ng, were spotted onto cotton swabs and subjected to sample processing by both methods. Higher DNA recovery rate was achieved for all three concentrations (5ng, 10ng and 20ng) using the new method, 32.9% vs 12.2%, 43.1% vs 14.9%, and 46.1% vs 21.5% respectively. It clearly demonstrates that more DNA can be recovered from touched objects using the new method.

Discussion

The increasing numbers of touch biological samples to be analysed demand fast methods, especially those that can consistently provide high reproducibility with minimal error. It is also critical to achieve high DNA recovery rate. Unfortunately, it is difficult to achieve both outcomes. Automated sample processing on a liquid workstation is the choice for fast and consistent sample processing. However, various problems interfering with DNA extraction and purification are often encountered. For example, in traditional workstations used for automatic biological detection, the robotic manipulator arms that transfer the DNA lysate are often blocked by the swabs. Thus the efficiency could be affected and the possible occurrence of cross-contamination could increase. We have been troubled with this problem for years, which urged us to make improvements.

The purpose of our study was thus to improve the workstation and make it more capable. In this paper, we report a design of a filtration plate and a high throughput sample processing method for touched objects. The new method improves profile obtained rate of touched objects from crime scenes.

Quantitative analysis by real-time PCR reveals higher DNA recovery with the new method. Thus a higher profile obtained rate of touch DNA collected at crime scene can be obtained using the new method. Since the amount of cell lysate transferred is one of the key points to improve efficiency of DNA extraction, we tried to combine 96-well filtration plates with TECAN Freedom EVO150-8 automated workstation. Centrifugation of 96-well filtration plate does help the transfer of more DNA lysate. As a result, DNA recovery is improved. At the same time, the new method also shortens sample processing time of up to an hour. While the plate specific to the workstation would cost more, it is more cost-effective as it can be easily used to deal with batches of samples, freeing up human labour.

In summary, the combined use of a 96-well filtration plate, centrifugation and an automatic liquid workstation not only improves profile obtained rate of touched objects, but also shortens sample processing time.

Figure legend:

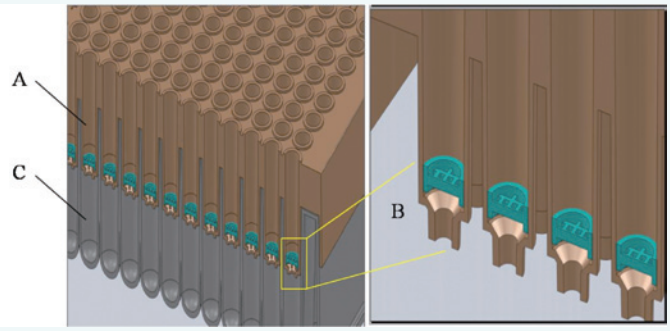

Figure 1: Illustrated structure of a 96-well centrifugal filtration plate (800μL cracking system, network format for carrier supporting, with microporous membrane filter). Swabs were added in part A, and Lysis buffer was then added into each well. The lysate can be transferred into part C through centrifugation, while swabs will not. The upper portion can be removed as a whole; the lower part can be put into the workstation for automatic extraction. The key part is part B, where there is network format for carrier supporting and microporous membrane filter.

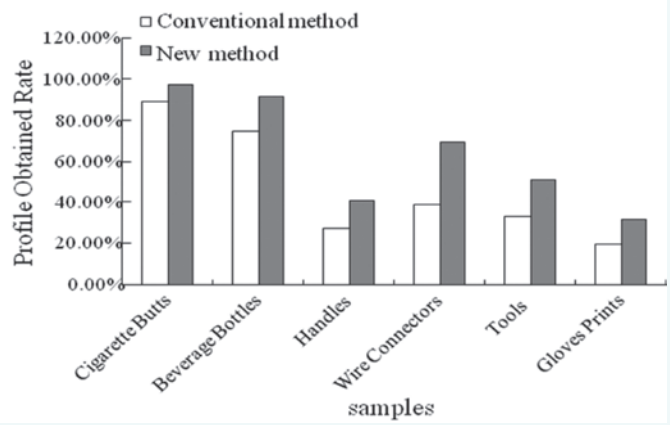

Figure 2: Profile obtained rate on various touched objects.

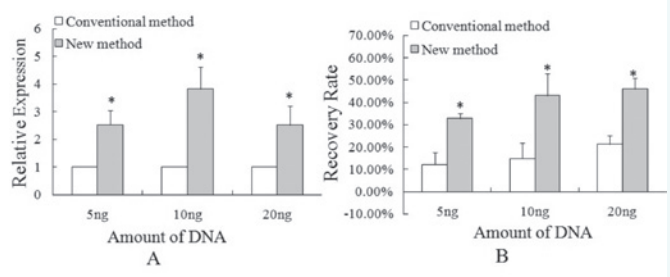

Figure 3: Quantitative analysis of DNA recovery by the two sample processing methods. Each bar represents mean ± SD from three samples ($p < 0.05$ vs. controls).*

Sample	Total	Detected	Not Detected	Profile Obtained Rate
Conventional method	536	231	305	43.10%
New method	553	301	252	54.43%
χ^2	13.992			
p-value	0.000			

Table 1: Profile obtained rate by conventional and new methods.

Sample	Cigarette Butts				Beverage Bottles				Handles			
	Total	Detected	Not Detected	Profile Obtained Rate	Total	Detected	Not Detected	Profile Obtained Rate	Total	Detected	Not Detected	Profile Obtained Rate
Conventional method	90	80	10	88.90%	43	32	11	74.40%	157	43	114	27.30%
New method	73	71	2	97.3%	22	20	2	90.9%	202	83	119	41.1%
χ^2	4.142				2.474				7.279			
p-value	0.083				0.213				0.007			

Sample	Wire Connectors				Tools				Gloves Prints			
	Total	Detected	Not Detected	Profile Obtained Rate	Total	Detected	Not Detected	Profile Obtained Rate	Total	Detected	Not Detected	Profile Obtained Rate
Conventional method	101	39	62	38.60%	63	21	42	33.30%	82	16	66	19.50%
New method	82	57	25	69.1%	76	39	37	51.3%	98	31	67	31.6%
χ^2	17.325				4.540				3.399			
p-value	0.000				0.033				0.065			

Table 2: Profile obtained rate of different touched objects by conventional and new methods.

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Interpretation Reliability of Contact DNA Mixed Profiles: An Assessment from Casework Contact Stains

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Abstract

DNA collected on handled objects, touched surfaces and worn clothes not attributed to any particular biological source such as blood, saliva, etc. could potentially contain amplifiable DNA which could be profiled. The transfer and persistence of DNA from such sources is uncertain and interpretation of profiles from contact stains thus requires caution. Of relevance to contact DNA mixtures, particularly in low template profiles, there is the inability to distinguish if the alleles were caused by indirect transfer (sporadic contamination) from the true alleles. Where the major contributor in a mixed contact profile can be inferred with no difficulty, the major source of the contact DNA can usually be reliably interpreted. The minor contributory sources, however, are mostly not amenable to reliable interpretation. This assessment study presents and considers the quality of mixed DNA profiles from casework contact stains, classifies the mixtures into three types, with no attempt to infer the mode of transfer.

Introduction

With the increasing sensitivity of DNA amplification kits and detection techniques, it is now possible to type DNA from a few cells deposited on handled objects, touched surfaces and worn clothes. Several different terms have been used to describe such DNA. The term 'touch DNA' has been used, but this can be misleading because it infers that the DNA recovered from a surface was present due to the surface being touched, but this is usually not known [1]. There is also wide usage of the term 'trace DNA', but this can have various meanings; it can refer to the amount of DNA present, the quality of DNA present, or to DNA detected by a LT-DNA technique [2]. In this assessment study, the term 'contact DNA' refers solely to DNA recovered from worn garments and accessories that cannot yet be attributed to an identifiable body fluid. The purpose of this assessment is to consider those items that yielded mixed DNA profiles, to evaluate the quality of the DNA profiles and to provide an interpretation guide as to the contributory source(s). There would be no attempt to infer the mode or likely mechanism of DNA transfer or the analysis of so-called 'wearer DNA' [1,3].

Materials and Methods

Contact stains were collected from garments and personal items (gloves and ski masks) submitted for forensic casework using one or two moistened swabs or with the double swab technique, using one wet swab followed by one dry swab [4]. Contact stains were collected from shirts and jackets at the collar and armhole regions; trousers, jeans and shorts at the interior waistband and the interiors of gloves and ski-masks.

DNA extraction was carried out with the standard phenol/chloroform protocol. STR amplification were carried out in 20 µl reaction volumes using 1 ng of DNA (if available) or the maximum possible with 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.

Table 1: Data from 68 casework contact DNA. N represents the number of samples for which the range of DNA concentration [DNA] in ng/µl and the mean was shown. The quality of DNA profiles obtained were categorised into Type I (major-minor distinguishable), Type II (major distinguishable but minor not reportable) and Type III (not reportable).

Item Type	N	[DNA]	Type I	Type II	Type III
Shirts	15	0.11 – 1.23 [0.53]	1	12	2
Jackets	9	0.06 – 0.89 [0.38]	3	4	2
Trousers	10	0.09 – 0.74 [0.25]	1	6	3
Jeans	12	0.18 – 1.36 [0.42]	2	7	3
Shorts	10	0.06 – 1.04 [0.60]	0	8	2
Gloves	8	0.48 – 1.25 [0.69]	2	4	2
Ski-masks	4	0.36 – 0.88 [0.51]	0	4	0

Results and Discussion

Mixed DNA profiles are commonly recovered from contact stains in casework items. Worn clothes or personal items are susceptible to indirect transfer of DNA (e.g. sneezing from passers-by), adventitious transfer from handling by non-wearers and drop-in contamination. In this study, the quality of mixed DNA profiles obtained were not particular to the type of garment or personal item. Hence, this assessment evaluates the mixed profiles obtained generally with no specific association made with the source item. Table 1 illustrates the types of casework items analysed, the amount of DNA recovered and the quality of the DNA profiles obtained. The assessment was based on 68 casework items where DNA profiles were successfully derived and were mixed profiles.

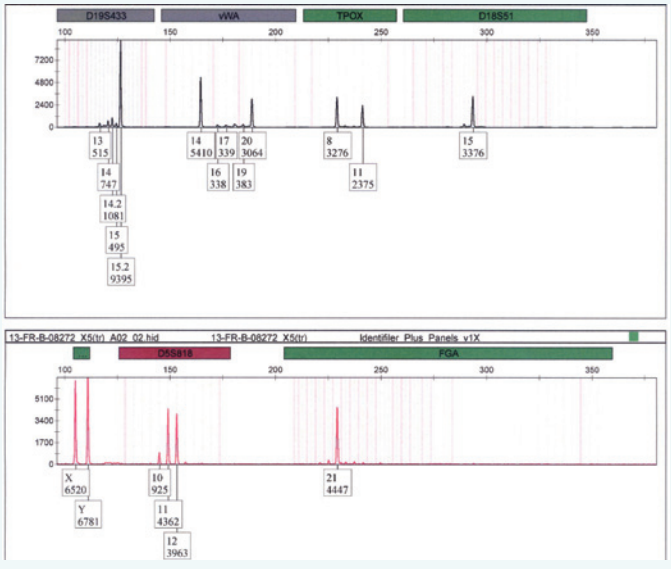

Figure 1: A typical contact mixture where the major contributor could be reliably interpreted.

From an assessment of 68 mixed DNA profiles derived from the contact stains of garments and personal items, 54 displayed mixtures where the major profile or contributor is distinguishable. Figure 1 depicts a typical contact mixture where the major profile could be reliably inferred. In all 54 profiles, the major profile was attributable to a reference DNA profile. The minor contributions in 45 of these 54 profiles were not reportable and were more consistent with being sporadic contamination than with being from true minor contributor(s). An important observation was that the major-minor ratio typically displayed by major-minor mixtures was consistently not observed for these 45 contact mixed profiles. From a review on the issues of DNA transfer on clothing items by Rudin and Inman [5], it was concluded that, "Where there is a known single habitual wearer, that person tends to be detected as the major source of DNA on a garment; minor profiles may also be detected from individuals with whom the habitual wearer has had close contact as well as from unknown sources." This demonstrates that DNA can be indirectly transferred to clothing but tends to be detected as the minor profile.

The remaining 14 mixtures in this assessment showed no distinguishable major profile; with 8 mixtures having more than six alleles at several loci in the multiplex and typically exhibited a 'laddering-like' effect at these loci (see Figure 2). Such mixtures are not reportable. It can be argued that with six alleles seen at any locus, the existence of multiple (≥3) contributory sources are assumed. In such a situation, all contributory sources could exhibit stochastic variations primarily caused by effects of low DNA template for any one source. Allele or locus dropouts are inevitable for any one contributory source. Further, with low template DNA profiles, sporadic contamination becomes important. Hence, the profile of any component contributor could not be reliably inferred.

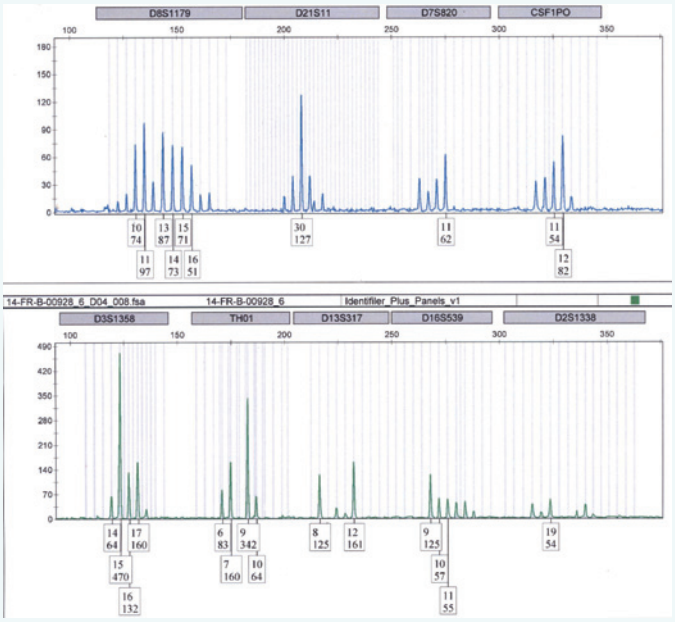

Figure 2: A contact mixture where the alleles or contributory sources are not reportable.

From the assessment of contact mixed profiles, a classification scheme can be devised where mixtures can be arbitrarily classified into Type I (major and minor contributors distinguishable), Type II (major contributor distinguishable but minor contributors not reportable) and Type III (contributors are not reportable) (Figure 3).

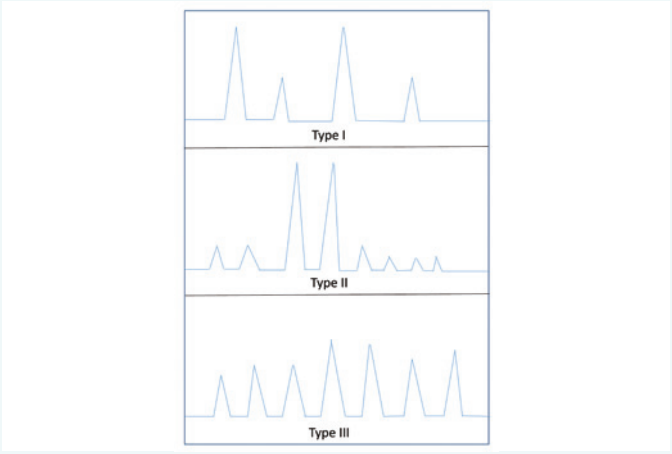

Figure 3: Schematic classification of the three types of contact mixtures observed.

The experimental data on DNA transfer as reviewed by Meakin and Jamieson [1] shows that neither the quantity of DNA recovered nor the quality of DNA profile obtained can be used to reliably infer the mode of transfer by which the DNA came to be on the surface of interest. Meakin and Jamieson [1] have also established that there is no strong correlation between a full or partial profile and the amount of DNA template at sub-optimal amounts of DNA. The poor correlation was also observed in this study where there were items with suboptimal amounts of DNA with reportable mixed profiles contrary to items with sufficient amounts of DNA that indicated mixed profiles which were not reportable. It was observed that the profile from a clothing item (shorts) having a DNA level of 0.06 ng/µl was reportable with an easily distinguishable major contributor. On the contrary, there were two other items of clothing (a jacket with 0.45 ng/µl of DNA and trousers with 0.39 ng/µl of DNA) and two gloves (with 0.53 and 0.72 ng/µl of DNA respectively) with seemingly sufficient amounts of DNA but yielded mixed profiles which were not reportable.

On the issues of DNA transfer, Rudin and Inman [5] have stated that forensic practitioners should resist pressure from the police, lawyers or even the Court, and only provide an opinion that is scientifically supported. Some forensic practitioners assert that an opinion can be given, whilst others do not. Hence, though forensic practitioners can reliably infer the source of the DNA in some contact stains, there is uncertainty as to the transfer and persistence of the DNA.

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Singapore Chinese, Malay and Indian Population Data for the PowerPlex® ESX17 System

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Abstract

Population allelic frequency data are necessary for the provision of statistical weight to the uniqueness of a DNA profile. Singapore is a melting pot of diverse ethnicity, with the Chinese, Malay and Indian populations respectively constituting 74%, 13%, and 9.1%, of the local populace [1]. In the present study, the allelic frequencies and forensic parameters of the 16 Short Tandem Repeat (STR) markers in the Promega PowerPlex® ESX 17 System were tested for the above populations to determine their suitability for forensic applications. With 16 STR markers, the combined random match probability is about 1.30 x 10^{-20} for the Chinese, 4.98 x 10^{-21} for Malay and 2.98 x 10^{-21} for Indian population in Singapore. This study is the first report on the allele frequencies of the D2S441, D1S1656, D10S1248, D12S391, D22S1045 and SE33 loci in the Singapore Chinese, Malay and Indian populations.

Introduction

The Promega PowerPlex® ESX 17 System is a 17 marker multiplex PCR kit designed according to recommendations by the European Network of Forensic Institutes (ENFSI) and European DNA Profiling (EDNAP) to include D2S441, D10S1248, D22S1045, D1S1656 and D12S391 on top of the seven European Standard Set (ESS) loci in 2006 [2]. With the exception of the highly polymorphic SE33, which is also co-amplified in this kit, these five new STR markers produce amplicons of less than 200 base-pairs (bp). These short fragments should also facilitate the recovery of DNA profiles from degraded samples [3]. The loci configuration of the Promega PowerPlex® ESX 17 System also allows for crosschecking for possible primer site variations at D3S1358, D8S1179, D18S51, D21S11, FGA, TH01, D19S433, D16S539, D2S1338 and vWA as compared to the Identifier® kit which is current used by the laboratory. As the discrimination power of a DNA profile for human identification is based on the statistical weight of STR loci in the population, the random match probability (RMP) generated from DNA profiling can be strengthened by increasing the number of polymorphic loci used [4]. The addition of STR markers would also facilitate kinship analyses.

Materials and Methods

A total of 334 Chinese, 282 Malay and 263 Indian individuals previously profiled from the Singapore population were utilised for this statistical analysis. All population data were sorted with Microsoft Excel and exported to the Promega PowerStats Microsoft Excel template to generate the respective allele frequencies and forensic parameters. The data were further analysed with the Arlequin 3.5.1.2 software for Hardy-Weinberg equilibrium (HWE) exact test using Markov Chain for all loci with forecasted chain length set at 1,000,000 along with 100,000 dememorisation steps [5]. Linkage between each loci pair in their respective population was also analysed.

Results and Discussion

Genotypes obtained from D3S1358, TH01, D21S11, D18S51, D2S1338 D16S539, vWA, D8S1179, FGA, and D19S433, were concordant with results previously obtained using AmpFISTR® Identifier® PCR amplification kit. The allele frequencies along with forensic parameters for all 16 STR loci were obtained for the Chinese, Malay and Indian population (Table 1 to Table 6). Table 7 tabulates the calculated combined random match probability, combined power of discrimination and combined probabilities of excluding paternity for each population achieved with all the STR markers. A total of 668, 564 and 526 alleles from the Chinese, Malay and Indian populations were examined respectively.

Exact test for HWE showed no significant departures (p-value > 0.05) for all but four loci from the populations analysed. These loci were examined and found to have incidences of rare alleles, i.e. less than five occurrences in each set of data. These rare alleles should pose little impact to statistical calculations as a minimum allele frequency (5/2n) can be applied to address such occurrences. It should also be noted that the HWE test is based on rejecting the null hypothesis when the likelihood of observation falls below 0.05. However, in multiple comparisons, the likelihood of observing rare events increases, which results in p-values falling below the statistical threshold. Such a phenomenon has also been reported by Budowle and colleagues [6]. Thus, the Bonferroni correction method was applied to loci with potential departures from HWE. The original p-values obtained were found to be significantly higher than the corresponding Bonferroni corrected p-values, suggesting no significant departures from HWE (data not shown). All markers were observed to be highly polymorphic with SE33 being the most informative and TH01 being the least informative across all populations.

Pair-wise linkage equilibrium test was performed using the Arlequin 3.5.1.2 software. Among the 120 loci pairs tested for each population, 4, 11 and 21 loci pairs from the Chinese, Malay and Indian populations respectively, were found to have p-values of less than 0.05, indicating possible linkage disequilibrium. The original p-values obtained were found to be significantly higher than the corresponding Bonferroni corrected p-values, which suggests no linkage disequilibrium (data not shown).

Loci D12S391 and vWA are located just 6.3 Mbp apart on chromosome 12 – both loci could be segregated together during recombination as revealed in a study by Budowle and colleagues in 2011 [7]. However, no evidence of linkage disequilibrium was detected in all three populations in our study. This was probably due to the lack of related individuals (father/son) in our data set. Hence, the multiplication of genotype frequencies is justified for these two loci in identity testing. The assumption of independence is, however, not valid during kinship analysis [7,8].

With these 16 STR markers, the combined RMP was calculated to be 1.3 x 10^{-20} for the Chinese, 4.98 x 10^{-21} for the Malay and 2.98 x 10^{-21} for the Indian populations in Singapore. The six STR markers (D2S441, D1S1656, D10S1248, D12S391, D22S1045 and SE33) would also further decrease the combined RMP by an order of 10^{-8} for each population when used together with the 15 autosomal STR markers from the Identifier® kit.

Table 1: Observed allele frequencies for vWA, TH01, FGA, D21S11, D19S433, D18S51, D16S539 and D8S1179 in the Singapore Chinese population.

Allele	vWA	TH01	FGA	D21S11	D19S433	D18S51	D16S539	D8S1179
6	-	0.0973	-	-	-	-	-	-
7	-	0.2680	-	-	-	-	-	-
8	-	0.0569	-	-	-	-	0.0015	0.0030
9	-	0.5030	-	-	-	0.0015	0.2575	-
9.3	-	0.0240	-	-	-	-	-	-
10	-	0.0479	-	-	-	0.0030	0.1332	0.1572
10.3	-	0.0015	-	-	-	-	-	-
11	-	0.0015	-	-	0.0030	0.0030	0.2560	0.1257
11.2	-	-	-	-	0.0030	-	-	-
12	-	-	-	-	0.0359	0.0419	0.2126	0.1317
12.2	-	-	-	-	0.0030	-	-	-
13	-	-	0.0045	-	0.2754	0.2021	0.1138	0.1572
13.2	-	-	-	-	0.0210	-	-	-
14	0.2725	-	-	-	0.2530	0.1886	0.0240	0.1826
14.2	-	-	-	-	0.1272	-	-	-
14.3	-	-	-	-	0.0015	-	-	-
15	0.0314	-	-	-	0.1003	0.1692	0.0015	0.1542
15.2	-	-	-	-	0.1287	-	-	-
16	0.1317	-	-	-	0.0120	0.1392	-	0.0689
16.2	-	-	-	-	0.0314	-	-	-
17	0.2620	-	0.0015	-	-	0.0928	-	0.0180
17.2	-	-	-	-	0.0015	-	-	-
18	0.1916	-	0.0284	-	0.0015	0.0449	-	0.0015
18.2	-	-	-	-	0.0015	-	-	-
19	0.0883	-	0.0524	-	-	0.0329	-	-
20	0.0210	-	0.0269	-	-	0.0254	-	-
21	0.0015	-	0.1138	-	-	0.0240	-	-
21.2	-	-	0.0015	-	-	-	-	-
22	-	-	0.1946	-	-	0.0120	-	-
22.2	-	-	0.0015	-	-	-	-	-
23	-	-	0.2290	-	-	0.0090	-	-
23.2	-	-	0.0120	-	-	-	-	-
24	-	-	0.1572	-	-	0.0045	-	-
24.2	-	-	0.0075	-	-	-	-	-
25	-	-	0.1078	-	-	0.0045	-	-
26	-	-	0.0389	0.0015	-	-	-	-
26.2	-	-	0.0015	-	-	-	-	-
27	-	-	0.0165	0.0045	-	-	-	-
27.2	-	-	0.0015	-	-	-	-	-
28	-	-	0.0015	0.0674	-	-	-	-
28.2	-	-	-	0.0030	-	-	-	-
29	-	-	-	0.2365	-	-	-	-
30	-	-	0.0015	0.2979	-	0.0015	-	-
30.2	-	-	-	0.0075	-	-	-	-
30.3	-	-	-	0.0015	-	-	-	-
31	-	-	-	0.0793	-	-	-	-
31.2	-	-	-	0.0614	-	-	-	-
32	-	-	-	0.0269	-	-	-	-
32.2	-	-	-	0.1632	-	-	-	-
33	-	-	-	0.0060	-	-	-	-
33.2	-	-	-	0.0374	-	-	-	-
34.2	-	-	-	0.0060	-	-	-	-
H_{obs}	0.8353	0.6677	0.8623	0.8413	0.8204	0.8563	0.7964	0.8563
H_{exp}	0.7950	0.6606	0.8554	0.8130	0.8156	0.8619	0.7929	0.8565
p-value$_{HWE}$	0.6879	0.6268	0.8318	0.8529	0.1221	0.0877	0.8976	0.6579
PD	0.9239	0.8363	0.9595	0.9364	0.9363	0.9633	0.9232	0.9595
PE	0.6662	0.3800	0.7192	0.6778	0.6374	0.7073	0.5924	0.7073
$PI_{typical}$	3.0364	1.5045	3.6304	3.1509	2.7833	3.4792	2.4559	3.4792

H_{obs}	Observed Heterozygosity
H_{exp}	Expected Heterozygosity
p-value$_{HWE}$	p-value obtained from HWE exact test
PD	Power of Discrimination
PE	Power of Exclusion
$PI_{typical}$	Typical Paternity Index

Table 2: Observed allele frequencies for D3S1358, D2S1338, SE33, D2S441, D1S1656, D10S1248, D12S391 and D22S1045 in the Singapore Chinese population.

Allele	D3S1358	D2S1338	SE33	D2S441	D1S1656	D10S1248	D12S391	D22S1045
8	-	-	-	-	-	0.0015	-	-
9.1	-	-	-	0.0165	-	-	-	-
10	-	-	-	0.2201	0.0015	0.0030	-	-
10.1	-	-	-	0.0015	-	-	-	-
11	-	-	-	0.3323	0.0689	0.0015	-	0.2141
11.2	-	-	0.0015	-	-	-	-	-
11.3	-	-	-	0.0823	-	-	-	-
12	-	-	0.0015	0.2051	0.0329	0.0659	-	0.0015
13	0.0030	-	0.0015	0.0180	0.1048	0.3488	-	0.0045
13.3	-	-	0.0015	-	-	-	-	-
14	0.0509	-	0.0015	0.1228	0.0853	0.2530	-	0.0374
14.3	-	-	0.0210	-	-	-	-	-
15	0.3219	-	-	0.0015	0.2859	0.2036	0.0165	0.2994
15.3	-	-	0.0359	-	0.0030	-	-	-
16	0.3159	0.0150	-	-	0.2500	0.1033	0.0030	0.2320
16.3	-	-	0.0689	-	0.0060	-	-	-
17	0.2530	0.0674	-	-	0.0674	0.0135	0.0763	0.1841
17.3	-	-	0.0539	-	0.0659	-	-	-
18	0.0494	0.1228	-	-	0.0075	0.0060	0.2365	0.0254
18.2	-	-	0.0704	-	-	-	-	-
18.3	-	-	-	-	0.0195	-	-	-
19	0.0060	0.1991	-	-	-	-	0.1841	0.0015
19.2	-	-	0.0629	-	-	-	-	-
19.3	-	-	-	-	0.0015	-	-	-
20	-	0.0988	-	-	-	-	0.1766	-
20.1	-	-	0.0075	-	-	-	-	-
20.2	-	-	0.0419	-	-	-	-	-
21	-	0.0434	-	-	-	-	0.1347	-
21.1	-	-	0.0254	-	-	-	-	-
21.2	-	-	0.0195	-	-	-	-	-
22	-	0.0554	-	-	-	-	0.0838	-
22.1	-	-	0.0479	-	-	-	-	-
22.3	-	-	0.0045	-	-	-	-	-
23	-	0.1841	0.0464	-	-	-	0.0464	-
23.2	-	-	0.0015	-	-	-	-	-
24	-	0.1332	0.0599	-	-	-	0.0269	-
25	-	0.0674	0.0524	-	-	-	0.0135	-
26	-	0.0120	0.0704	-	-	-	0.0015	-
27	-	0.0015	0.0599	-	-	-	-	-
28	-	-	0.0704	-	-	-	-	-
28.3	-	-	0.0614	-	-	-	-	-
30	-	-	0.0569	-	-	-	-	-
31	-	-	0.0389	-	-	-	-	-
32	-	-	0.0075	-	-	-	-	-
33	-	-	0.0030	-	-	-	-	-
34	-	-	0.0030	-	-	-	-	-
36	-	-	0.0015	-	-	-	-	-
H_{obs}	0.7156	0.8653	0.9311	0.8084	0.7784	0.7335	0.8623	0.7934
H_{exp}	0.7286	0.8708	0.9474	0.7778	0.8236	0.7588	0.8459	0.7759
p-value$_{HWE}$	0.8446	0.2179	0.6867	0.2378	0.3820	0.1350	0.7566	0.0071
PD	0.8777	0.9669	0.9920	0.9136	0.9484	0.9027	0.9561	0.9070
PE	0.4528	0.7252	0.8594	0.6147	0.5597	0.4820	0.7192	0.5869
$PI_{typical}$	1.7579	3.7111	7.2609	2.6094	2.2568	1.8764	3.6304	2.4203

Table 3: Observed allele frequencies for vWA, TH01, FGA, D21S11, D19S433, D18S51, D16S539 and D8S1179 in the Singapore Malay population.

Allele	vWA	TH01	FGA	D21S11	D19S433	D18S51	D16S539	D8S1179
6	-	0.1152	-	-	-	-	-	-
7	-	0.2535	-	-	-	-	-	-
8	-	0.1064	-	-	-	-	0.0142	0.0018
8.3	-	0.0035	-	-	-	-	-	-
9	-	0.3511	-	-	0.0035	-	0.1702	-
9.3	-	0.0904	-	-	-	-	-	-
10	-	0.0798	-	-	-	0.0018	0.2021	0.0833
11	-	-	-	-	0.0018	-	0.2748	0.1028
11.2	-	-	-	-	0.0018	-	-	-
12	-	-	-	-	0.0461	0.0745	0.1915	0.0922
12.2	-	-	-	-	0.0053	-	-	-
13	-	-	0.0018	-	0.2234	0.1046	0.1348	0.1933
13.2	-	-	-	-	0.0426	-	-	-
14	0.2074	-	0.0018	-	0.2340	0.1862	0.0124	0.1986
14.2	-	-	-	-	0.1046	-	-	-
15	0.0532	-	-	-	0.0975	0.2323	-	0.2163
15.2	-	-	-	-	0.2004	-	-	-
16	0.1489	-	-	-	0.0089	0.1986	-	0.0780
16.2	-	-	-	-	0.0230	-	-	-
17	0.2518	-	0.0018	-	0.0053	0.0745	-	0.0284
17.2	-	-	-	-	0.0018	-	-	-
18	0.1950	-	0.0035	-	-	0.0496	-	0.0035
19	0.1259	-	0.0638	-	-	0.0319	-	-
19.2	-	-	0.0018	-	-	-	-	-
20	0.0177	-	0.0550	-	-	0.0177	-	-
21	-	-	0.1525	-	-	0.0142	-	-
21.2	-	-	0.0177	-	-	-	-	-
22	-	-	0.2270	-	-	0.0035	-	-
22.2	-	-	0.0195	-	-	-	-	-
23	-	-	0.1436	-	-	0.0053	-	-
23.2	-	-	0.0089	-	-	-	-	-
24	-	-	0.1436	-	-	0.0018	-	-
24.2	-	-	0.0089	-	-	-	-	-
24.3	-	-	0.0018	-	-	-	-	-
25	-	-	0.0869	-	-	0.0035	-	-
26	-	-	0.0514	-	-	-	-	0.0018
27	-	-	0.0089	0.0053	-	-	-	-
28	-	-	-	0.0567	-	-	-	-
29	-	-	-	0.2358	-	-	-	-
30	-	-	-	0.2429	-	-	-	-
30.2	-	-	-	0.0372	-	-	-	-
31	-	-	-	0.1046	-	-	-	-
31.2	-	-	-	0.0922	-	-	-	-
31.2	-	-	-	0.0319	-	-	-	-
32.2	-	-	-	0.1365	-	-	-	-
33	-	-	-	0.0035	-	-	-	-
33.2	-	-	-	0.0390	-	-	-	-
34.2	-	-	-	0.0089	-	-	-	-
36.1	-	-	-	0.0018	-	-	-	-
37.2	-	-	-	0.0035	-	-	-	-
H_{obs}	0.8546	0.7837	0.8794	0.8759	0.8440	0.8404	0.8085	0.8121
H_{exp}	0.8158	0.7747	0.8673	0.8420	0.8316	0.8474	0.8009	0.8450
p-value$_{HWE}$	0.2218	0.0717	0.8179	0.4633	0.7810	0.4215	0.5927	0.3895
PD	0.9327	0.9144	0.9550	0.9523	0.9487	0.9576	0.9269	0.9550
PE	0.7040	0.5691	0.7536	0.7393	0.6830	0.6761	0.6149	0.6216
$PI_{typical}$	3.4390	2.3115	4.1471	3.9167	3.2045	3.1333	2.6111	2.6604

Table 4: Observed allele frequencies for D3S1358, D2S1338, SE33, D2S441, D1S1656, D10S1248, D12S391 and D22S1045 in the Singapore Malay population.

Allele	D3S1358	D2S1338	SE33	D2S441	D1S1656	D10S1248	D12S391	D22S1045
8	-	-	-	-	0.0018	-	-	-
9	-	-	0.0018	0.0035	-	-	-	-
9.1	-	-	-	0.0071	-	-	-	-
10	-	-	-	0.1791	0.0035	-	-	-
11	-	-	0.0018	0.3191	0.1046	0.0018	-	0.2110
11.3	-	-	-	0.1738	-	-	-	-
12	-	-	0.0018	0.1312	0.0461	0.0621	-	0.0018
12.3	-	-	-	0.0018	-	-	-	-
13	0.0071	-	-	0.0142	0.0798	0.3316	-	0.0053
14	0.0496	-	0.0035	0.1560	0.1259	0.2287	-	0.0319
14.3	-	-	-	-	0.0018	-	-	-
15	0.2677	0.0018	0.0106	0.0106	0.2145	0.2234	0.0071	0.3812
15.3	-	-	0.0018	-	0.0142	-	-	-
16	0.3582	0.0089	0.0390	0.0018	0.2411	0.1188	0.0035	0.1702
16.3	-	-	-	-	0.0177	-	-	-
17	0.2270	0.1028	0.0461	0.0018	0.0656	0.0301	0.0638	0.1826
17.3	-	-	-	-	0.0390	-	0.0018	-
18	0.0816	0.0816	0.0479	-	0.0195	0.0035	0.2252	0.0142
18.2	-	-	0.0018	-	-	-	-	-
18.3	-	-	0.0745	-	0.0195	-	0.0018	-
19	0.0053	0.2323	-	-	0.0053	-	0.1897	0.0018
19.1	-	-	0.0018	-	-	-	-	-
20	0.0035	0.1099	0.0319	-	-	-	0.1596	-
20.2	-	-	0.0071	-	-	-	-	-
21	-	0.0177	0.0372	-	-	-	0.1099	-
21.2	-	-	0.0177	-	-	-	-	-
22	-	0.0638	0.0071	-	-	-	0.1223	-
22.2	-	-	0.0177	-	-	-	-	-
22.3	-	-	0.0018	-	-	-	-	-
23	-	0.1826	0.0018	-	-	-	0.0674	-
23.2	-	-	0.0567	-	-	-	-	-
24	-	0.1277	-	-	-	-	0.0337	-
24.2	-	-	0.0195	-	-	-	-	-
25	-	0.0567	-	-	-	-	0.0089	-
25.2	-	-	0.0372	-	-	-	-	-
26	-	0.0124	-	-	-	-	0.0035	-
26.2	-	-	0.1170	-	-	-	-	-
27.2	-	-	0.1170	-	-	-	-	-
28	-	0.0018	-	-	-	-	0.0018	-
28.2	-	-	0.1241	-	-	-	-	-
28.3	-	-	0.0018	-	-	-	-	-
29.2	-	-	0.0621	-	-	-	-	-
30	-	-	0.0018	-	-	-	-	-
30.2	-	-	0.0408	-	-	-	-	-
31.2	-	-	0.0248	-	-	-	-	-
32	-	-	0.0018	-	-	-	-	-
32.2	-	-	0.0319	-	-	-	-	-
33	-	-	0.0035	-	-	-	-	-
33.2	-	-	0.0035	-	-	-	-	-
34.2	-	-	0.0018	-	-	-	-	-
H_{obs}	0.7128	0.8582	0.9007	0.79433	0.8440	0.7731	0.8653	0.7411
H_{exp}	0.7406	0.8608	0.9319	0.79535	0.8549	0.7703	0.8524	0.7479
p-value$_{HWE}$	0.8168	0.3449	0.0161	0.0720	0.6044	0.1986	0.7139	0.6619
PD	0.8921	0.9602	0.9870	0.9238	0.9588	0.9080	0.9576	0.8942
PE	0.4483	0.7110	0.7969	0.5886	0.6830	0.5500	0.7251	0.4947
$PI_{typical}$	1.7407	3.5250	5.0357	2.4310	3.2045	2.2031	3.7105	1.9315

Table 5: Observed allele frequencies for vWA, TH01, FGA, D21S11, D19S433, D18S51, D16S539 and D8S1179 in the Singapore Indian population.

Allele	vWA	TH01	D8S1179	D21S11	D19S433	D18S51	D16S539	D8S1179
5	-	0.0019	-	-	-	-	-	-
6	-	0.2205	-	-	-	-	-	-
7	-	0.1768	-	-	-	-	-	-
8	-	0.1616	-	-	-	-	0.0760	0.0038
9	-	0.3137	-	-	0.0019	-	0.1445	0.0019
9.3	-	0.1084	-	-	-	-	-	-
10	0.0019	0.0171	-	-	-	0.0152	0.1065	0.1768
11	-	-	-	-	-	0.0209	0.3346	0.0875
12	-	-	-	-	0.0608	0.0494	0.1939	0.0970
12.2	-	-	-	-	0.0152	-	-	-
13	0.0076	-	-	-	0.3251	0.1236	0.1255	0.1274
13.2	-	-	-	-	0.0228	-	-	-
14	0.1502	-	-	-	0.2319	0.2814	0.0171	0.1844
14.2	-	-	-	-	0.0646	-	-	-
15	0.0741	-	-	-	0.1141	0.1730	0.0019	0.1958
15.2	-	-	-	-	0.0913	-	-	-
16	0.2243	-	-	-	0.0456	0.1464	-	0.0951
16.1	-	-	-	-	0.0190	-	-	-
17	0.2681	-	0.0019	-	0.0038	0.0722	-	0.0304
17.2	-	-	-	-	0.0038	-	-	-
18	0.1768	-	0.0133	-	-	0.0475	-	-
19	0.0760	-	0.0741	-	-	0.0399	-	-
20	0.0190	-	0.1350	-	-	0.0228	-	-
20.2	-	-	0.0019	-	-	-	-	-
21	0.0019	-	0.1711	-	-	0.0019	-	-
21.1	-	-	0.0019	-	-	-	-	-
21.2	-	-	0.0038	-	-	-	-	-
22	-	-	0.1331	-	-	0.0019	-	-
22.2	-	-	0.0019	-	-	-	-	-
23	-	-	0.1711	-	-	0.0019	-	-
24	-	-	0.1464	-	-	0.0019	-	-
24.2	-	-	0.0038	-	-	-	-	-
25	-	-	0.0684	-	-	-	-	-
25.1	-	-	0.0019	-	-	-	-	-
25.2	-	-	0.0038	-	-	-	-	-
26	-	-	0.0475	-	-	-	-	-
26.2	-	-	0.0038	0.0133	-	-	-	-
27	-	-	0.0114	-	-	-	-	-
28	-	-	0.0038	0.1350	-	-	-	-
28.2	-	-	-	0.0019	-	-	-	-
29	-	-	-	0.1920	-	-	-	-
29.2	-	-	-	0.0038	-	-	-	-
29.3	-	-	-	0.0057	-	-	-	-
30	-	-	-	0.1863	-	-	-	-
30.2	-	-	-	0.0247	-	-	-	-
31	-	-	-	0.0456	-	-	-	-
31.2	-	-	-	0.1179	-	-	-	-
32	-	-	-	0.0152	-	-	-	-
32.2	-	-	-	0.1863	-	-	-	-
33.2	-	-	-	0.0684	-	-	-	-
34.2	-	-	-	0.0019	-	-	-	-
35.1	-	-	-	0.0019	-	-	-	-
H_{obs}	0.8061	0.7491	0.9164	0.8441	0.7909	0.8061	0.7529	0.8365
H_{exp}	0.8138	0.7850	0.8729	0.8554	0.8096	0.8431	0.7979	0.8548
p-$value_{HWE}$	0.8956	0.3958	0.2768	0.7081	0.5708	0.4064	0.2842	0.0242
PD	0.9378	0.9205	0.9579	0.9598	0.9382	0.9578	0.9299	0.9579
PE	0.6104	0.5081	0.8289	0.6833	0.5822	0.6104	0.5147	0.6684
$PI_{typical}$	2.5784	1.9924	5.9773	3.2073	2.3909	2.5784	2.0231	3.0581

Table 6: Observed allele frequencies for D3S1358, D2S1338, SE33, D2S441, D1S1656, D10S1248, D12S391 and D22S1045 in the Singapore Indian population.

Allele	D3S1358	D2S1338	SE33	D2S441	D1S1656	D10S1248	D12S391	D22S1045
8	-	-	0.0095	-	0.0437	-	-	-
9	-	-	-	0.0019	0.0038	-	-	-
10	-	-	-	0.3289	0.0019	-	-	0.0019
11	-	-	-	0.4144	0.1692	0.0057	-	0.3384
11.3	-	-	-	0.0665	-	-	-	-
12	-	-	0.0152	0.0684	0.0837	0.0190	-	-
12.2	-	-	0.0019	-	-	-	-	-
12.3	-	-	-	0.0057	-	-	-	-
13	-	0.0019	0.0190	0.0057	0.1616	0.1711	-	0.0114
13.2	-	-	0.0019	-	-	-	-	-
13.3	-	-	0.0019	-	-	0.0019	-	-
14	0.0418	-	0.0133	0.0817	0.0932	0.2091	-	0.0418
14.2	-	-	0.0019	-	-	-	-	-
14.3	-	-	0.0019	-	-	-	-	-
15	0.3061	0.0095	0.0114	0.0209	0.1198	0.3194	0.0019	0.3840
15.2	-	-	0.0038	-	-	-	-	-
15.3	-	-	0.0095	-	0.0228	-	-	0.0019
16	0.3289	0.0019	0.0532	0.0057	0.1483	0.2091	0.0095	0.1426
16.1	-	-	0.0019	-	-	-	-	-
16.3	-	-	0.0038	-	0.0285	-	-	-
17	0.2281	0.0608	0.0646	-	0.0703	0.0570	0.1236	0.0608
17.2	-	-	-	-	-	-	0.0019	-
17.3	-	-	-	-	0.0209	-	-	-
18	0.0894	0.1673	0.0760	-	0.0133	0.0076	0.2414	0.0152
18.3	-	-	-	-	0.0152	-	0.0152	-
19	0.0038	0.1882	0.1103	-	-	-	0.1597	0.0019
19.2	-	-	0.0019	-	-	-	0.0038	-
19.3	-	-	-	-	0.0038	-	0.0019	-
20	0.0019	0.0684	0.0760	-	-	-	0.1369	-
20.1	-	-	0.0038	-	-	-	-	-
20.2	-	-	0.0019	-	-	-	-	-
21	-	0.0342	0.0228	-	-	-	0.0837	-
21.1	-	-	0.0038	-	-	-	-	-
21.2	-	-	0.0076	-	-	-	-	-
22	-	0.0760	0.0114	-	-	-	0.0951	-
22.1	-	-	0.0038	-	-	-	-	-
22.2	-	-	0.0171	-	-	-	-	-
23	-	0.2091	0.0057	-	-	-	0.0837	-
23.2	-	-	0.0304	-	-	-	-	-
24	-	0.1084	0.0019	-	-	-	0.0266	-
24.2	-	-	0.0285	-	-	-	-	-
25	-	0.0627	0.0019	-	-	-	0.0152	-
25.2	-	-	0.0418	-	-	-	-	-
26	-	0.0114	-	-	-	-	-	-
26.2	-	-	0.0304	-	-	-	-	-
27	-	-	0.0019	-	-	-	-	-
27.2	-	-	0.0456	-	-	-	-	-
28	-	-	0.0019	-	-	-	-	-
28.2	-	-	0.0418	-	-	-	-	-
29.2	-	-	0.0513	-	-	-	-	-
30.2	-	-	0.0684	-	-	-	-	-
31.2	-	-	0.0342	-	-	-	-	-
32.2	-	-	0.0247	-	-	-	-	-
33	-	-	0.0019	-	-	-	-	-
33.2	-	-	0.0266	-	-	-	-	-
34	-	-	0.0019	-	-	-	-	-
34.2	-	-	0.0057	-	-	-	-	-
35.2	-	-	0.0019	-	-	-	-	-
H_{obs}	*0.7453*	*0.8517*	*0.9430*	*0.6692*	*0.8327*	*0.7871*	*0.8517*	*0.7072*
H_{exp}	*0.7377*	*0.8633*	*0.9507*	*0.7051*	*0.8859*	*0.7790*	*0.8595*	*0.7132*
p-$value_{HWE}$	*0.5591*	*0.6233*	*0.1867*	*0.0165*	*0.7353*	*0.8441*	*0.8147*	*0.9338*
PD	*0.8792*	*0.9636*	*0.9915*	*0.8678*	*0.9740*	*0.9140*	*0.9627*	*0.8688*
PE	*0.5017*	*0.6982*	*0.8837*	*0.3822*	*0.6611*	*0.5753*	*0.6982*	*0.4395*
$PI_{typical}$	*1.9627*	*3.3718*	*8.7667*	*1.5115*	*2.9886*	*2.3482*	*3.3718*	*1.7078*

Table 7: Calculated combined random match probability, combined power of discrimination and combined probabilities of excluding paternity achieved with 16 STR loci included in the PowerPlex® ESX17 System for the Singapore Chinese, Malay and Indian populations.

	Chinese	Malay	Indian
Combined random match	-	0.0019	-
Probability	1.30×10^{-20}	4.98×10^{-21}	2.98×10^{-21}
Combined power of discrimination	>0.9999999999	>0.9999999999	>0.9999999999
Combined probability of excluding paternity	0.9999999490	0.9999999676	0.9999999285

Conclusion

In conclusion, the present study has shown the alleles of these 16 STR loci to be in HWE, with no linkage disequilibrium between the loci. The loci and the presented allele frequencies are suitable for application to forensic casework.

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Application of Routine Methodology in Chemical Profiling of Heroin Seized from Northern and Central Region of Peninsular Malaysia

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Abstract

This research was conducted in order to discriminate chromatographic profiles of 53 heroin samples seized in two regions, Kuala Lumpur and Penang, in Peninsular Malaysia. Using a routine method in sample preparation, major alkaloids, adulterant and impurities were analysed and separated by gas chromatographic technique, and identified by mass spectrometry (GCMS) using HP-5 column. Peak area ratios of the components were evaluated in this study. A method based on Pearson's correlation coefficient was chosen to compare heroin chemical profiles. This evaluation was performed on the variables (processed from the peak area) to investigate the separation of the samples/chromatographic profiles by degree of similarity between different seizures of illicit drugs. Classification techniques such as hierarchical cluster analysis (HCA) and principal component analysis (PCA) of the components demonstrated the presence of at least 2 groups within the seized heroin samples.

Introduction

The different components found in the analysed illicit drugs matrices allow the characterisation of a link between two illicit drug products. The characterisation of major and minor components in the drug samples confiscated on different occasions and/or in different places provides unique pieces of evidence in order to prove the relationships between samples particularly at the source level. A variety of sophisticated analytical techniques have been described in the literature, especially gas chromatography, which gives a high resolution in the separation of impurities as well as good sensitivity and reproducibility [1].

Over the years, many works related to heroin profiling have been published. The profiling methods utilised in the published work are tedious in terms of sample preparation and analytical methods [2,3]. For a high volume casework laboratory, which fully occupies the instruments for casework samples, a routine method for chemical profiling is recommended. Heroin profiling based upon presence of major and minor components can provide useful information in criminal investigation and specifically on drug sources of supply and relationships between seizures. Many papers have been published to assist the forensic chemist in establishing the degree of similarity between different seizures of illicit drugs. As suggested by Besacier *et al*. [4], opium alkaloids, adulterants, diluents and trace impurities in heroin samples are the important components that should be profiled. Esseiva *et al*. [5] established links between illicit heroin samples by using a combination of principal component analysis (PCA) and calculation of a correlation value between samples. In 1988, Barnfield *et al*. developed a method which depends on simple and straightforward sample pretreatment, followed by gas chromatography analyses for the routine profiling of illicit heroin samples [6]. Herein, we have developed a method of analyses able to give a sufficient chemical profile of heroin for comparison in routine use: it includes major alkaloids, impurities and cutting agents. The advantage of this method allows major impurities to be detected in one single analysis as well as the amount of diacetylmorphine and the identification of both adulterants and diluents present in the matrix.

Most heroin seizures made by the Royal Malaysian Police officers at the northern part of Peninsular Malaysia are of South-East Asia origin and principally involve Burma, Laos and Thailand. Another source of heroin to Malaysia is from South-West Asia, which today refers mainly to Afghanistan and Pakistan. These heroin seizures were made by the Royal Malaysian Police officers at the central region of Malaysia [7]. In this study, analyses of the major alkaloids and adulterants by GCMS technique were performed on the seized heroin samples. Pattern recognition techniques were applied to the processed GCMS data. This study proposes a discussion on the application of routine sample preparation and analysis method that provided meaningful discrimination of 53 samples seized in two regions of Peninsular Malaysia and allows discrimination/ identification of linkages between samples.

Materials and Methods

For this research, seized heroin samples were collected from two laboratories. Fifteen samples were received from Penang laboratory (Northern region) and thirty-eight samples were from headquarters (Central region).

For analysis, major alkaloids and adulterants were extracted from 20 - 60 mg (based on typical purity of heroin samples seized in Malaysia) of the powdered heroin hydrochloride. The sample was weighed in a 5 mL GC vial. The sample was dissolved in the internal standard solution containing 0.18 mg/mL 2,2,2 triphenyl acetophenone in 1:9 methanol:chloroform mixture, made up to the mark and ultrasonicated for a few minutes. The aliquots were analysed by gas chromatography mass spectrometry (GCMS) using narscan method (routine drug method for full scan analysis). Narscan method is a method routinely used in our laboratory in order to obtain the full chromatography of drug sample. The method has been validated with major alkaloids (codeine, morphine, acetylcodeine, 6-monoacetylmorphine, heroin and noscapine) and adulterants (caffeine).

An Agilent 6890 GC and 5973 mass selective detector (MSD) were used with a non-polar column (HP-5), 30 m x 0.25 mm i.d., film thickness 0.25 µm; the oven temperature programme started at 80 ºC for 2 min and then increased at:
20°C /min to 150°C & hold for 2 min
10°C /min to 280°C & hold for 5 min
20°C /min to 300°C & hold for 14 min

The injector and detector (transfer line) temperatures were set at 250 and 300ºC respectively; helium was used as a carrier gas at a constant flow rate of 2 mL/min; 1µL of extract was injected in the split mode (40:1).

Results and Discussion

After evaluation of the profile of each of the 53 heroin samples analysed for major alkaloids and adulterants, ten components (meconin, theophylline, caffeine, methorphan, codeine, morphine, acetylcodein, 6-monoacetylmorphine, heroin and noscapine) were selected in this study (Figure 1 and 2). To compare correlation values between the profiles, Pearson correlation coefficients were calculated for the data set. The Pearson correlation coefficients were calculated for

each pair of samples using variables. The peak areas were normalised to the sum of the peak area of the 10 components. The ratio areas were pre-treated with the weight taken and all the 10 variables in a single data statistically analysed. As a set point, the 95.00 threshold value was chosen across the data set to facilitate linkage of all samples to their possible linkages (similar samples). Accurate discrimination by chromatographic profiles of the 53 samples was achieved using the components from this study normalised to the sum of the components and pre-treated by weight taken. The lowest coefficient calculated for a pair of samples from within a region was 95.93 and the maximum threshold that would allow the 53 samples within each region to be deemed similar was 99.99.

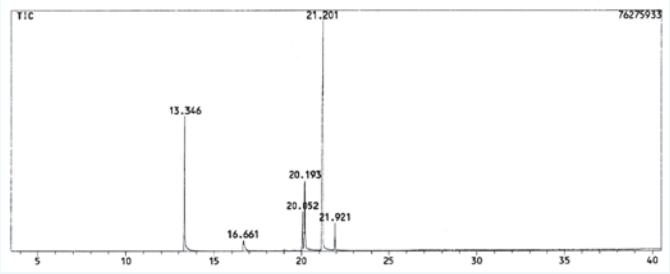

Figure 1: Expanded GC Chromatogram of a typical heroin sample from Central region. Peak caffeine (13.3 min), methorphan (16.6 min), acetylcodeine (20.0 min), 6-monoacetylmorphine (20.1 min), heroin (21.2 min), IS (21.9 min)

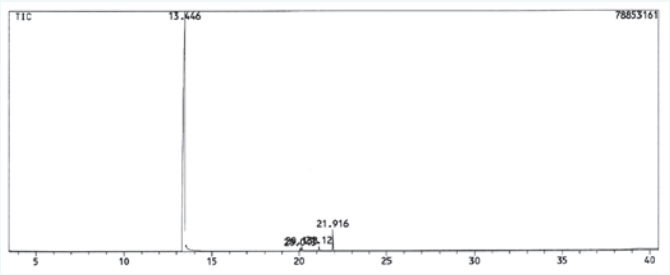

Figure 2: Expanded GC Chromatogram of a typical heroin sample from Northern region. Peak caffeine (13.4 min), acetylcodeine (20.0 min), 6-monoacetylmorphine (20.1 min), heroin (21.2 min), IS (21.9 min)

Pattern recognition technique

HCA was performed using SPSS software version 18.0 with 10 variables on the data set. Two clustering methods, nearest and further neighbour, and two distance measures, Euclidean and squared Euclidean, were examined for the data set. In total, four combinations of the clustering and distance methods were used for each identified data set as follows:

1. nearest neighbour, Euclidean distance;
2. nearest neighbour, squared Euclidean distance;
3. furthest neighbour, Euclidean distance; and
4. furthest neighbour, squared Euclidean distance.

As demonstrated in the previous profiling study [8], variables functioned effectively in grouping all the related samples into their respective distribution links on a dendogram, when using furthest neighbour, Euclidean distance. The dendrogram produced using furthest neighbour and Euclidean distance of GCMS data set is displayed in Figure 3.

PCA has been used in similar published works, but in this study, the validity of the resulting clusters can be assessed because the samples are from 2 known sources/origins. Remarkably, PCA of the 10 components data allows the samples to be grouped correctly according to geographical origin (Figure 1 and 2). PCA was performed using SPSS 18.0, the correlation matrix option was selected since the variables were measured on different scales, and the varimax rotation method was utilised in order to minimise the number of variables with high loadings on each factor.

In the resulting principal components (PCs), samples that were found to be most similar were clustered within it. Figure 4 and 5 show plots of clusters by 2 and 3 principal components respectively. PCA generated two clusters in the data, each one corresponding to a geographical origin. Evaluation of central region cluster showed the presence of one northern region sample (purple spot) to be clustered in this group (Figure 4 and 5). Besides that, one dissimilar sample origin was also found within the central region (yellow spot with purple circle in Figure 4 and 5). This sample might be from different source/production batch. Furthermore with the presence of meconin, this sample showed significant differences in the chromatographic profiles compared to other central region samples. By this algorithm, accurate discrimination of the two groups of samples (of different chromatographic profiles) was possible when 10 components were included in target data.

Conclusion

This work has reported the major alkaloids and adulterants found in 53 seized heroin samples. Routine method in sample preparation was used and the components were separated and analysed by GCMS using HP-5 column. There were distinct differences in profiles of 53 seized heroin samples received from two laboratories. Evaluation with statistical software, allowed the clustering of the seized samples into two groups, with a high degree of certainty as HCA and PCA gave the same decision. Pearson correlation coefficients and HCA with PCA have proven that accurate discrimination between chromatographic profiles of heroin samples is achievable when targeting components data from GCMS results using narscan method (routine drug method for full scan analysis) is considered.

Figure 3: Plot resulting from HCA shows blue circle (Northern region), and red and purple circle for (Central region).

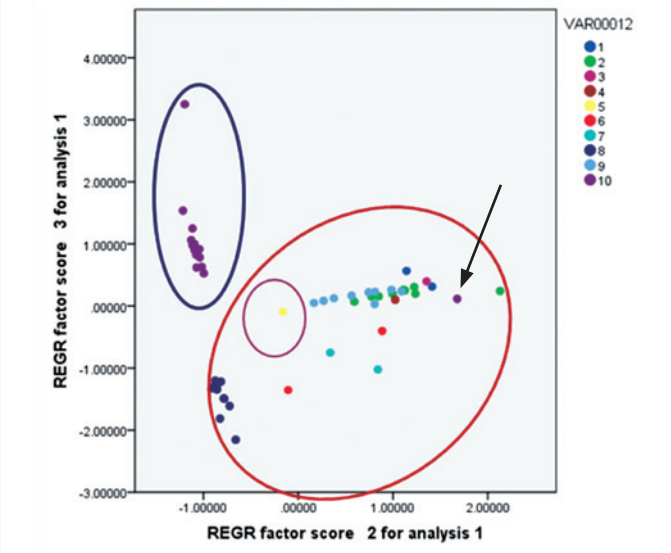

Figure 4: Plot resulting from PCA (2 PCs evaluation) shows blue circle (Northern region) and red circle for (Central region).

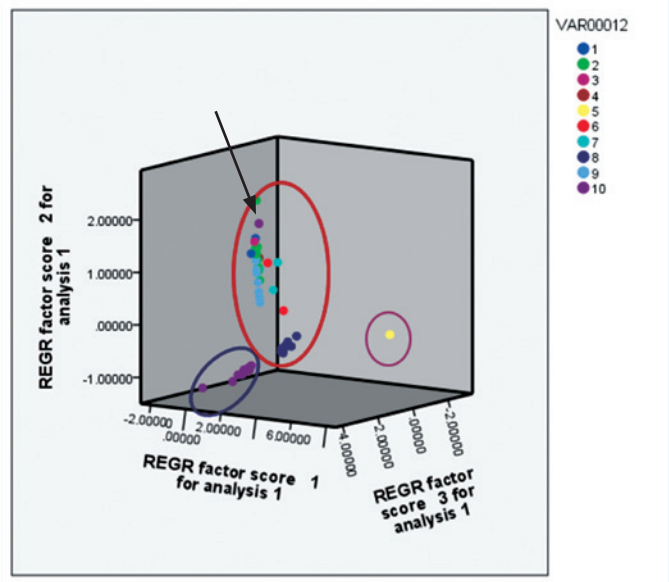

Figure 5: Plot resulting from PCA (3 PCs evaluation) shows blue circle (Northern region) and red circle for (Central region).

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Identification of 3-Trifluoromethylphenylpiperazine (TFMPP) from the Unknown White Crystalline Substance

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Abstract

3-Trifluoromethylphenylpiperazine (TFMPP) was detected recently by the drug laboratory of IFS (Institute of Forensic Science, Ministry of Public Security, P. R. China). The white crystalline substance was delivered by the police to our laboratory. Preliminary screening of the white crystalline substance was performed using colour tests. The presence of TFMPP was confirmed by GC-MS and NMR. This is the first report of this substance in Mainland China. It is suggested that more attention should be paid to the piperazine family of new psychoactive substances in Asia.

3-Trifluoromethylphenylpiperazine (TFMPP)

TFMPP is a recreational drug of the piperazine chemical class. TFMPP is a central nervous system stimulant, which has been reported to stimulate the release of dopamine, noradrenaline, and serotonin, and also inhibit their re-uptake. Thus TFMPP is an amphetamine mimic and is predominantly found in tablets either as the main component or in combination with other piperazines, amphetamine, cocaine, ketamine or MDMA. It is sold as a legal alternative to the illicit drug MDMA ("Ecstasy") under the name "Legal X" [1]. The structure of TFMPP is shown in Figure 1.

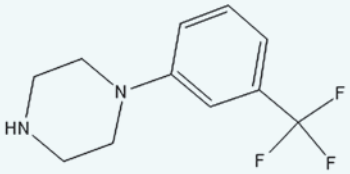


Figure 1: Chemical structure of TFMPP.

Preliminary Screening – Colour Tests [2]

Colour tests are often non-specific in nature and serve to include (or exclude) the presence of a broad range of compounds.

Marquis Test:

One drop of 40% formaldehyde solution was mixed with 1 mL of concentrated sulphuric acid. 3 mg of the test sample was placed on a spot plate depression and 3 drops of the mixed reagents were added.

Simon's Test:

3 mg of the test sample was placed on a spot plate depression and 1 drop of 20% aqueous sodium carbonate solution was added. Next an equivalent amount of 50% ethanolic acetaldehyde solution followed by 1% aqueous sodium nitroprusside was added.

Dragendorff Test:

1 g of bismuth subnitrate was dissolved in a small amount of concentrated HCl. 25% aqueous ammonia was added drop-wise until no more precipitate was formed. The precipitate was filtered and preserved, washed with water and then dissolved in 1 mL of concentrated HCl. A solution of 3 g potassium iodide in 1 mL water was prepared and added to the precipitate solution. 48 mL of 70% aqueous acetic acid was added to the resulting solution. The test sample was placed on a spot plate depression and 3 drops of the reagent was added.

For the Marquis test, a white to pale brown precipitate was observed. For the Simon's test, no colour change was observed when 3 mg of the test sample was used but a blue colour was observed when 10 mg of the test sample was used. A combination of the Marquis reagent with the Simon's reagent may be effective in distinguishing some piperazines from methamphetamine or MDMA while in the field. For 1-benzylpiperazine (BZP)-like compounds, the Marquis reagent shows negative results or faint colouration. The reagent produces a strong red-orange colour with amphetamines, while MDMA-type compounds produce a blue-black colour.

For the Simon's test, no colour change was observed for some piperazines while a blue colour was observed for methamphetamine or MDMA when 3 mg of the test sample was used.

For the Dragendorff test, a red precipitate was observed. In Dragendorff test, an orange, red-orange, or brown-orange precipitate suggests the presence of an alkaloidal base and a strong positive result suggests the presence of tertiary amines. Hence piperazines can be distinguished from methamphetamine or MDMA through colour tests.

Definitive Identification by Gas Chromatography–Mass Spectrometry (GC-MS) and Nuclear Magnetic Resonance (NMR)

GC-MS [3]

GC-MS is one of the most commonly used techniques for the identification of forensic drug samples.

GC-MS (EI) analysis condition is as follows: Agilent GC-MS 7890/ 5975 C, column DB-5 MS 30 m × 0.25 mm × 0.25 μm, temperature: column 60ºC (0 min) with 15ºC/min to 300ºC (15 min); injector: 280ºC; split ratio: 20:1; transfer line: 250ºC; ion source: 230ºC; ionization energy: 70 eV; injection volume: 1 μL; Helium gas with flow: 1.0 mL/min; Scan parameter: 40-500 m/z.

The TFMPP drug standard was purchased from Cerilliant, with a concentration of 1.0 mg/mL in methanol.

Approximately 10 mg of the white crystalline substance was dissolved in 10 mL methanol and then subjected to GC-MS analysis. The mass spectrum is shown in Figure 2. TFMPP in the white crystalline substance was identified by GC-MS with characteristic fragmentation ions of 56, 145, 172, 188 and 230. The sample and the TFMPP drug standard have the same retention time of 8.9 min and the same characteristic fragmentation ions.

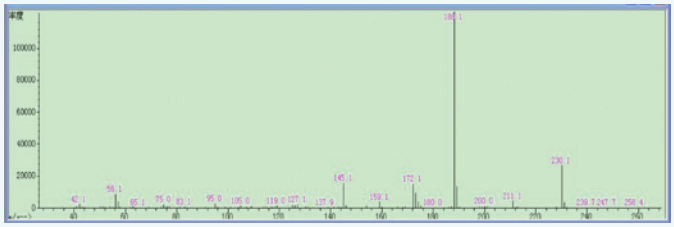


Figure 2: GC-MS spectrum of the crystalline substance.

^{1}H and ^{13}C NMR

The sample was dissolved in refluxing methanol, followed by vacuum filtration to obtain the purified substance. The crystalline substance was in the hydrochloride form.

^{1}H and ^{13}C NMR spectra were recorded in 5 mm NMR tubes on a Varian Inova 600 spectrometer on solutions in Methanol-d_4. Chemical shifts were reported in parts per million (ppm).

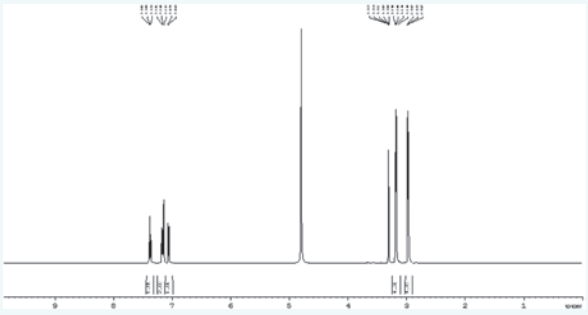


Figure 3: ^{1}H NMR spectrum of the crystalline substance.

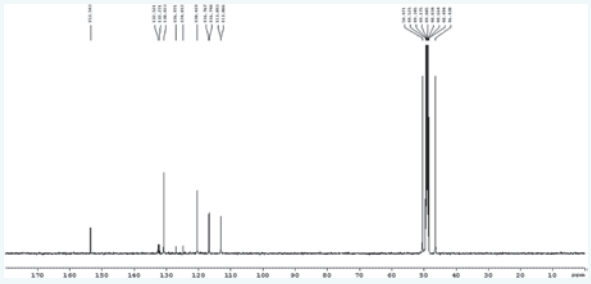


Figure 4: ^{13}C NMR spectrum of the crystalline substance.

Table 1: Data of ^{1}H NMR.

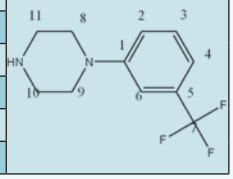
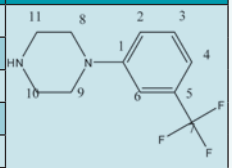
Proton	Chemical shift (ppm), integral, multiplicity and coupling constant (Hz, where applicable)	Structure assignment
H-2	7.18, 1H, d, 8.4 Hz	
H-3	7. 39, 1H, t, 8.0 Hz	
H-4	7.07, 1H, d, 7.7 Hz	
H-6	7.15, 1H, s	
H-8\9	3.31, 4H, m	
H-10\11	2.98, 4H, m	

Table 2: Data of ^{13}C NMR.

Carbon	Chemical shift (ppm), multiplicity and coupling constant (Hz, where applicable)	Structure assignment
C-1	153.54, s	
C-2	120.42, s	
C-3	130.82, s	
C-4	117.26, s	
C-5	132.40, q, 31.7 Hz	
C-6	113.08, s	
C-7	125.91, q, 271.6 Hz	
C-8\9	50.47, s	
C-10\11	46.44, s	

From the ^{1}H and ^{13}C NMR spectra data, we can conclude the structure of the crystalline substance.

Conclusion

This is the first time our laboratory has detected TFMPP. In this case study, GC-MS and NMR were used for identification of TFMPP in the white crystalline substance received in our laboratory.

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The Identification of New Psychoactive Substances (NPS) in "Happy Water" and "Coffee Powder"

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Introduction

The United Nations Office on Drugs and Crime (UNODC) defines New Psychoactive Substances (NPS) as "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". It is noted that the term 'new' does not necessary mean that the substance is newly invented but it refers to those that have been misused recently [1]. In response to the emerging NPS in Macau, the anti-drug law was amended earlier this year to place generic control on the derivatives of synthetic cathinones, synthetic cannabinoids and piperazines. A summary of the control of piperazines, synthetic cathinones, synthetic cannabinoids and the generic definitions under the anti-drug law in Macau is presented in Table 1. The psychoactive plant, *Salvia divinorum* together with its active ingredient, Salvinorin A, have also been listed in the controlled drug schedule.

In Macau, NPS are generally encountered as a constituent in "Happy water" – liquid drug mixture commonly sealed in small glass bottles or packaged as oral solution of Chinese medicine (Figure 1). To a lesser extent, NPS have also been encountered as a constituent in mixture with instant milk tea or coffee powder marketed as "Milk tea powder" or "Coffee powder" (Figure 2). The appearance of "Happy water" and "Coffee powder" in the illicit drug market in Macau can be dated back to 2010 and 2011 respectively. These new drug types are abused by mixing with other beverages, making them very popular at entertainment places. Most of the "Happy water" and "Coffee powder" samples submitted to our laboratory are found to contain multiple substances and the composition is diverse - ranging from illicit drugs, medicinal substances to flavouring agents.

Piperazines	-1-Benzylpiperazine is listed as a controlled drug. -Other derivatives are controlled by the following generic definition: "Any compound structurally derived from 1-benzylpiperazine or 1-phenylpiperazine by modification in any of the following ways: (1) by substitution at the second nitrogen atom of the piperazine ring with alkyl, benzyl, haloalkyl or phenyl groups; (2) by substitution in the aromatic ring to any extent with alkyl, alkoxy, alkylenedioxy, halide or haloalkyl groups."
Synthetic cathinones	Cathinoines are controlled by the generic definitions as follow: -"Any compound structurally derived from 2-amino-1-phenyl-1-propanone by modification in any of the following ways: (1) by 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; (2) by substitution at the 3-position with an alkyl substituent; (3) by substitution at the nitrogen atom with alkyl or dialkyl groups, or by inclusion of the nitrogen atom in a cyclic structure." -"Any compound structurally derived from 2-aminopropan-1-one by substitution at the 1-position with any monocyclic, or fused-polycyclic ring system (not being a phenyl ring or alkylenedioxyphenyl ring system), whether or not the compound is further modified in any of the following ways: (1) by substitution in the ring system to any extent with alkyl, alkoxy, haloalkyl or halide substituents, whether or not further substituted in the ring system by one or more other univalent substituents; (2) by substitution at the 3-position with an alkyl substituent; (3) by substitution at the 2-amino nitrogen atom with alkyl or dialkyl groups, or by inclusion of the 2-amino nitrogen atom in a cyclic structure."
Synthetic cannabinoids	-Naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone is listed as a controlled drug. -Other classes of synthetic cannabinoids are controlled by the following generic definitions: Naphthoylindoles: "Any compound structurally derived from 3-(1-naphthoyl)indole or 1H-indol-3-yl-(1-naphthyl)methanone by substitution at the nitrogen atom of the indole ring by alkyl, alkenyl, cycloalkylmethyl, cycloalkylethyl or 2-(4-morpholinyl)ethyl, whether or not further substituted in the indole ring to any extent and whether or not substituted in the naphthyl ring to any extent" Naphthoylpyrroles: "Any compound structurally derived from 3-(1-naphthoyl)pyrrole by substitution at the nitrogen atom of the pyrrole ring by alkyl, alkenyl, cycloalkylmethyl, cycloalkylethyl or 2-(4-morpholinyl)ethyl, whether or not further substituted in the pyrrole ring to any extent and whether or not substituted in the naphthyl ring to any extent." Naphthylmethylindenes: "Any compound structurally derived from 1-(1-naphthylmethyl)indene by substitution at the 3-position of the indene ring by alkyl, alkenyl, cycloalkylmethyl, cycloalkylethyl or 2-(4-morpholinyl)ethyl, whether or not further substituted in the indene ring to any extent and whether or not substituted in the naphthyl ring to any extent." Phenylacetylindoles: "Any compound structurally derived from 3-phenylacetylindole by substitution at the nitrogen atom of the indole ring with alkyl, alkenyl, cycloalkylmethyl, cycloalkylethyl or 2-(4-morpholinyl)ethyl, whether or not further substituted in the indole ring to any extent and whether or not substituted in the phenyl ring to any extent." Cyclohexylphenols: "Any compound structurally derived from 2-(3-hydroxycyclohexyl)phenol by substitution at the 5-position of the phenolic ring by alkyl, alkenyl, cycloalkylmethyl, cycloalkylethyl or 2-(4-morpholinyl)ethyl, whether or not further substituted in the cyclohexyl ring to any extent."

Table 1: A summary of the control of piperazines, synthetic cathinones and synthetic cannabinoids and the generic definitions under the anti-drug law of Macau.

In this article, the analytical data of some of the seized "Happy water" and "Coffee powder" samples that are determined to contain NPS will be presented.

Figure 1: Image of "Happy water".

Figure 2: Image of "Milk tea powder".

Materials and Methods

Sample Preparation

For the liquid sample, 5 mL was extracted at pH 3, 7 and 11, each with 5 mL of chloroform. The chloroform layers collected were mixed and evaporated to dryness under nitrogen. The residue was reconstituted in methanol for GC/MS analysis.

For the powder sample, 10 mL of 1:1 ethanol:chloroform (v/v) was added to approximately 1 g of sample. After a brief sonication, the extract was evaporated to dryness under nitrogen and reconstituted in methanol for GC/MS analysis.

Instrumentation

Agilent 7890A GC coupled with 5975C mass-selective detector with HP-5MS column (30 m x 0.25 mm x 0.25 µm);
GC condition:

- Injection volume = 1 µL
- Split mode (15:1)
- Inlet temperature = 250ºC
- Carrier gas = helium, flow rate = 1 mL/min
- Temperature programme: initial temperature = 100ºC; hold for 0 min; ramp to 280ºC (12ºC/min) and hold for 8 min.
- Total run time = 23 min

MSD condition:

- EI mode, voltage = 70 eV
- Scan range = 40-550 amu
- Source temperature = 230ºC

Results and Discussion

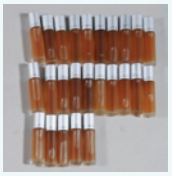
	Sample	Package	Substances Detected
1	Brown liquid		*Controlled:* TFMPP, MDMA , methamphetamine, ketamine, nimetazepam
			Uncontrolled: Paracetamol, antipyrine, amantadine, caffeine, chlorpheniramine, tramadol, guaifenesin
2	Brown liquid		*Controlled:* Ketamine, 2C-B, MDMA, methamphetamine, DBZP, BZP
			Uncontrolled: Caffeine, chlorpheniramine, guaifenesin, phenazepam
3	Brown liquid		Controlled: Methylone, piperonal
			Uncontrolled: Phenazepam
4	Cream-coloured powder		Controlled: Ketamine, methamphetamine, cocaine, pFPP, TFMPP
			Uncontrolled: Caffeine
5	Pale brown powder		Controlled: Methylone
			Uncontrolled: Phenazepam, caffeine

Table 2: Composition of the "Happy water" and "Coffee powder" samples.

The substances detected in the five "Happy water" and "Coffee powder" samples are presented in Table 2 and their corresponding total ion chromatographs are shown in Figure 3 to 7.

TFMPP (1-(3-trifluoromethylphenyl)piperazine), DBZP (1,4-dibenzylpiperazine), BZP (1-benzylpiperazine), pFPP (para-fluorophenylpiperazine) and methylone were identified in the samples and their mass spectra are presented in Figure 8 to 12 respectively. In Sample 1, 2 and 4, the NPS were present in a mixture with other illicit drugs e.g. methamphetamine, ketamine, and cocaine. In Sample 3 and 5, methylone was determined as the main constituent and no other illicit drugs were detected except that in Sample 3, piperonal was present which may be used as a precursor for methylone synthesis [2]. The result shows that these samples were adulterated with other substances such as amantadine, caffeine, guaifenesin, phenazepam, paracetamol and tramadol. Guaifenesin is an expectorant in cough syrup preparations and Qian *et al* [3] suggested that guaifenesin was added in "Happy water" with the intention to mask the presence of drugs by the odour of cough syrup. It is noted that phenazepam, a long-acting benzodiazepine which is not listed as a controlled drug in the UN conventions nor in Macau, was identified in three of the five samples. Since 2013, our laboratory has encountered phenazepam as a substitute of nimetazepam in Erimin 5 pills, as well as in "Happy water" and "Coffee powder".

Conclusion

This paper demonstrated the analytical data of five "Happy water" and "Coffee powder" samples containing NPS. TFMPP, DBZP, BZP, pFPP and methylone, were identified by GC-MS and their corresponding mass spectra were presented. Due to the limited data available, lack of reference standards and the structural similarity between NPS analogues, NPS have

posed challenges on the chemical analysis of drugs. “Happy water” and “Coffee powder” have not only provided a more hidden way to distribute NPS and other illicit drugs, but also further complicated the analysis due to their diversity and complexity in composition. Exchange of information regarding emerging NPS and the analytical data between forensic drug testing laboratories is very valuable in order to conquer these challenges.

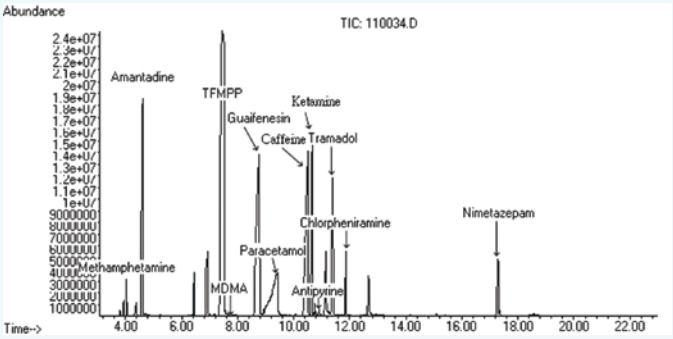


Figure 3: TIC of Sample 1.

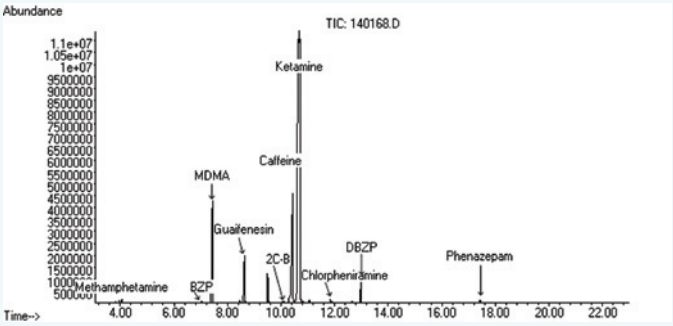


Figure 4: TIC of Sample 2.

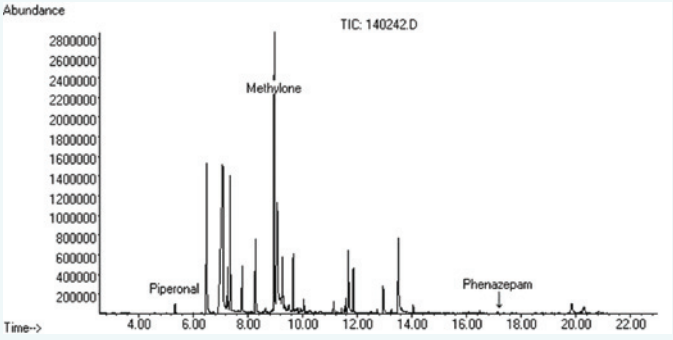


Figure 5: TIC of Sample 3.

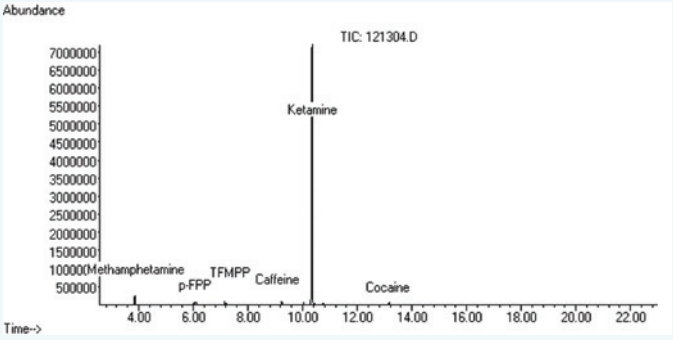


Figure 6: TIC of Sample 4.

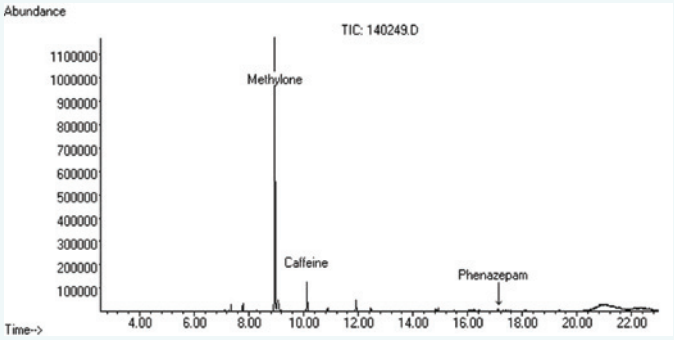


Figure 7: TIC of Sample 5.

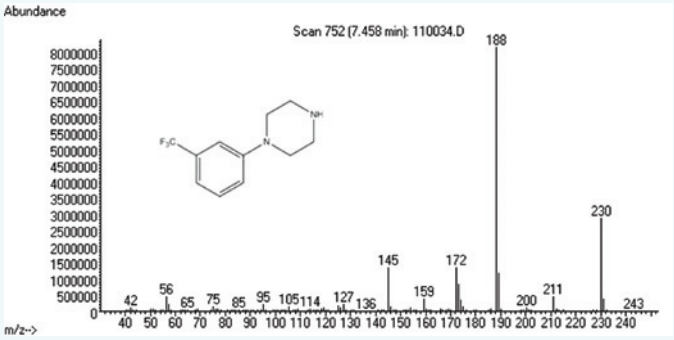


Figure 8: Mass spectrum of TFMPP.

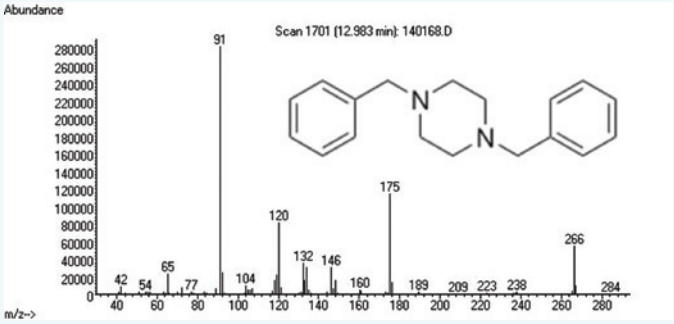


Figure 9: Mass spectrum of DBZP.

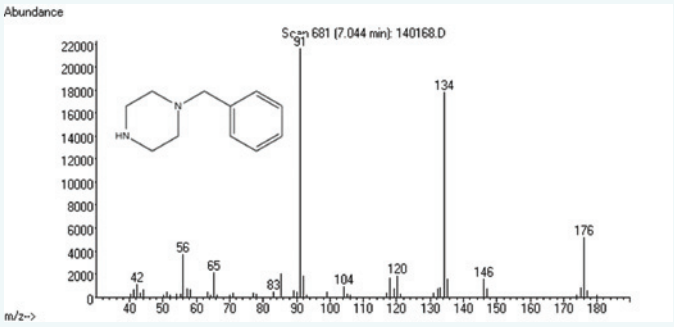


Figure 10: Mass spectrum of BZP.

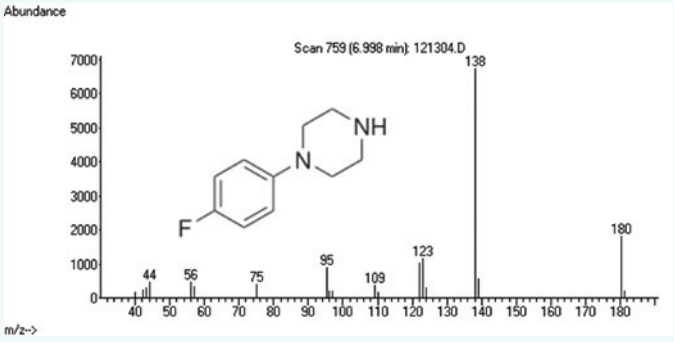


Figure 11: Mass spectrum of pFPP.

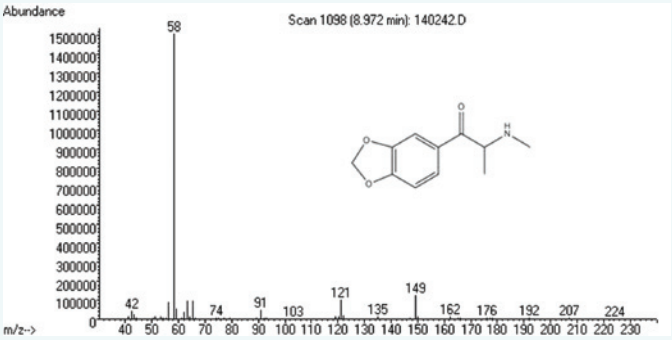


Figure 12: Mass spectrum of methylone.

References

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2. Kavanagh P, O'Brien J, Fox J, *et al*. The analysis of substituted cathinones. Part 3. Synthesis and characterisation of 2,3-methylenedioxy substituted cathinones. *Forensic Sci Int.*. 2012; 216:19-28.
3. Qian ZH, Chang Y, XU P, *et al*. Analysis of "happy water" samples. *Chinese Journal of Drug Abuse Prevention and Treatment.* 2011; 17(4):239-242.

Simultaneous Determination of 10 Synthetic Cannabinoids in Novel "Spice" Drugs by HPLC and HPLC-MS/MS

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Introduction

Synthetic cannabinoids and cannabimimetics, commonly referred to as synthetic cannabinoids, are compounds in herbal products marketed as "Spice" and act as the agonists of cannabinoid receptors CB1 and/or CB2. In many cases, they are significantly more potent than tetrahydrocannabinol (THC, the principal psychoactive constituent of cannabis plant) [1]. Most of them are developed by scientists for high therapeutic activity without large side effects, but in recent years, underground laboratories have developed these compounds as marijuana substitutes for illicit use [2]. Along with the non-controlled status, these compounds are rapidly and widely publicised by drug-using communities, resulting in explosive growth in the use of "synthetic cannabinoids" type products.

The first published methods applied to the analysis of synthetic cannabinoids in plant materials used LC or GC-MS [3]. In China, this is the first report of analysis of synthetic cannabinoids by HPLC-MS/MS, and the first report of simultaneous determination of more than 2 kinds of synthetic cannabinoids using HPLC.

Materials and Methods

Chemicals and Reagents

Chromatographic grade methanol and acetonitrile were purchased from Thermo Fisher (USA). Chromatographic grade formic acid and ammonium formate (99% pure) were purchased from Fluka (Switzerland). Synthetic cannabinoid standards were all purchased from Cerilliant (USA).

Instrumentation

The HPLC system comprised of a Shimadzu Prominence HPLC (Japan), including LC-20AD XR pumps and controlled by a LC-solution software. A Shim-pack XR-ODS C18 (4.6 × 250 mm, 5 µm) column was used at 45ºC. The mobile phase system contained methanol-acetonitrile (50:50, v/v) and water, and the percentage of organic phase changed between 66% and 89.1% in a linear gradient elution of 33 min. Samples were detected by an ultraviolet detector at 220 nm.

The HPLC-MS/MS system comprised of an Agilent 1290 Infinity HPLC connected to 6460 Triple Quad mass spectrometer (USA). A Poroshell 120 EC-C18 (3.0 × 50 mm, 2.7 µm) column was used at 35ºC. The mobile phase consisted of methanol and water. The percentage of organic phase changed between 70% and 90% in a linear gradient elution in 12 min.

Sample Preparation

Samples were dissolved using methanol, centrifuged for 10 min and filtered through 0.22 µm membrane filter.

Results and Discussion

Method Development

Nine kinds of solvents were chosen to dissolve samples and extract analytes. Methanol was the best and water was the worst.

Table 1: HPLC-MS/MS parameters for synthetic cannabinoids.

Analyte (CAS#)	Precursor ion	Product ion	Fragmentor (V)	CE (V)	ESI mode
JWH-018(209414-07-3)	342.2	**155.0**	135	28	positive
		127.0		50	
JWH-073(208987-48-8)	328.2	**155.0**	135	26	positive
		127.0		50	
JWH-122(619294-47-2)	356.2	**169.0**	145	30	positive
		141.0		50	
JWH-147(914458-20	382.2	**155.0**	125	22	positive
		127.0		70	
JWH-200(103610-04-4)	385.2	**155.0**	130	25	positive
		114.2		30	
JWH-203(864445-54-5)	340.1	**125.0**	130	30	positive
		188.0		20	
JWH-250(864445-43-2)	336.2	**121.0**	125	18	positive
		200.2		26	
Hu-210(112830-95-2)	385.3	**367.3**	150	30	negative
		301.1		40	
CP47497(70434-82-1)	317.3	**299.3**	150	26	negative
		245.3		35	
CP47497(C8) (70434-92-3)	331.3	**313.3**	150	26	negative
		259.3		35	

**Bold masses depict quantification transitions.*

In the HPLC-MS/MS method, the function of "time segment" and "MRM segment" made it possible to use different polarity modes in different time segments without decreasing sensitivity. As for the mobile phase, methanol was chosen as the organic phase and four kinds of aqueous phases were tested (Table 2). To gain the greatest response, we chose water, which would make the method more convenient and sensitive. Besides, all four aqueous phases had similar intra-day relative standard deviations (RSDs).

Method Validation

Validation of the HPLC method included the assessment of selectivity, linearity (1 to 100 µg/mL, with coefficients > 0.99999), limits of detection (0.1~0.5 µg/mL), imprecision (intra-day RSDs ≤ 1.68%, inter-day RSDs ≤ 2.36%) and accuracy (98.15%~102.07% of target concentration).

Validation of the HPLC-MS/MS method included the assessment of selectivity, linearity (1 to 100 or 10 to 1000 ng/mL, with coefficients > 0.999), limits of detection (0.05~0.5 ng/mL), imprecision (intra-day RSDs ≤ 3.26%, inter-day RSDs ≤ 7.68%) and accuracy (96.55%~103.27% of target concentration). Positive identification of the drugs was based on the maximum permitted tolerances for relative abundance of the precursor/product ion pairs under the guidelines established by the European Union Decision 2002/657/EC.

To test the selectivity of the two methods, natural marijuana samples spiked with 10 synthetic cannabinoids were analyzed. Figures 1 and 2 show 10 synthetic cannabinoids completely separated from each other and the main components of marijuana.

Table 2: Peak heights of 10 synthetic cannabinoids when using different aqueous mobile phase.

Analyte	Ion transition	Peak height			
		Water	0.2% HCOOH	5mM $HCOONH_4$	0.2% HCOOH -5mM $HCOONH_4$
JWH-200	385.2--->155.0	4083	990	2916	3396
JWH-250	336.2--->121.0	8805	2772	4935	4683
JWH-073	328.2--->155.0	6105	2085	5376	5007
JWH-203	340.1--->125.0	3246	1083	3432	3243
JWH-018	342.2--->155.0	6072	2127	5622	5301
JWH-122	356.2--->169.0	7710	3033	6330	6105
CP47497	317.3--->299.3	560	90	110	30
JWH-147	382.2--->155.0	5544	2190	8373	8097
CP47497(C8)	331.3--->313.3	610	110	150	40
Hu-210	385.3--->367.3	320	130	110	40

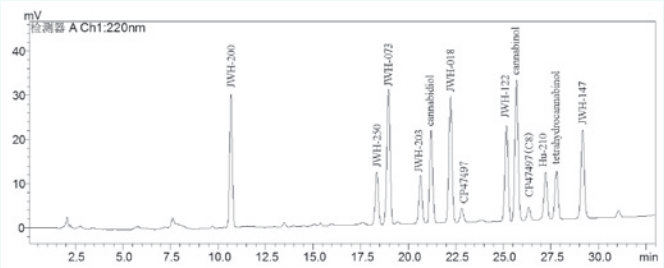

Figure 1: Chromatogram of natural marijuana spiked with 10 synthetic cannabinoids (by HPLC).

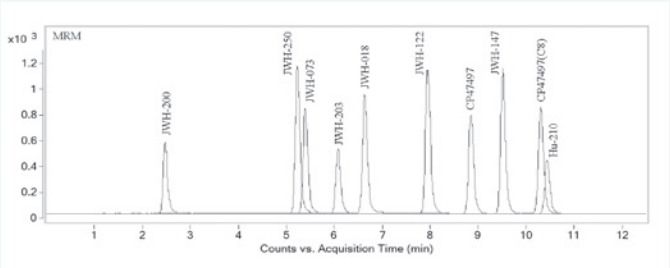

Figure 2: MRM chromatograph of natural marijuana spiked with 10 synthetic cannabinoids (by HPLC-MS/MS).

Conclusion

Taking into account the high amounts of synthetic cannabinoids found in seized novel "spice" drugs during recent years, this study established HPLC and HPLC-MS/MS methods are capable of simultaneous determination of common synthetic cannabinoids. Both methods were applied to real cases and proved to be accurate, precise, sensitive and fast. This study provides a reliable reference for qualitative and quantitative analysis of synthetic cannabinoids in novel drug samples.

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Case Study: Differentiation of Three Fluoroethcathinone Positional Isomers

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Introduction

New Psychoactive Substances (NPS) are "substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat" as defined by the United Nations Office on Drugs and Crime (UNODC) [1]. The UNODC further clarified that the term 'new' does not necessarily refer to new inventions but to substances that have recently become available. These include synthetic drugs that are able to bind to the same biological receptors that the existing banned drugs are binding to, e.g. JWH-015 binding to cannabinoid receptors [2], hence inducing comparable psychoactive effects. Many legislations are also struggling to keep up with the multitudes of NPS that emerge within a short period of time [3]. For the drug testing laboratories, the similarities in the structures of some NPS pose challenges in the analysis as they often possess similar physical and chemical properties. This is especially evident in positional isomers, and it has been suggested that it is one of the most challenging area in separation science [4]. The separation and positive identification of these positional isomers is hence of great importance in the analysis of these types of exhibits.

Our laboratory routinely screens all drug samples using the in-house gas chromatographic method employing a HP 5-MS (12.5 m × 0.25 mm × 0.25 µm) column. This column was chosen because of its good performance in providing sufficient resolution (Rs ≥ 1.5) of the common drugs of abuse (e.g. diamorphine, methamphetamine and ketamine) encountered in Singapore to positively identify them within a short runtime of 17.5 minutes. In addition, successful separation and identification were also achieved in the analysis of many groups of NPS positional isomers. One such example is the 2-, 3-, and 4- positional isomers of methoxymethcathinone which have similar MS fragmentation patterns and therefore, it is not possible to differentiate one isomer from another solely based on their fragmentation pattern (Figure 1). The identification of these isomers based on retention time is hence important. However, not all groups of NPS positional isomers can be separated using this in-house GC method, one example being 2-, 3-, and 4-fluoroethcathinone (FEC) positional isomers. A search in the literature showed that FEC positional isomers can be discriminated by product ion spectrometry [5], whereby unique product ions were generated for each FEC isomer by chemical ionisation (CI) with methane gas under GC–MS condition with detection by triple quadrupole mass spectrometer.

In this study, we present alternative methods of differentiating FEC isomers, namely chromatographic differentiation by gas chromatography (GC) and liquid chromatography (LC). Both GC and LC instruments were coupled to a mass spectrometer where ions were produced via electron impact (EI) and electrospray ionisation (ESI) respectively.

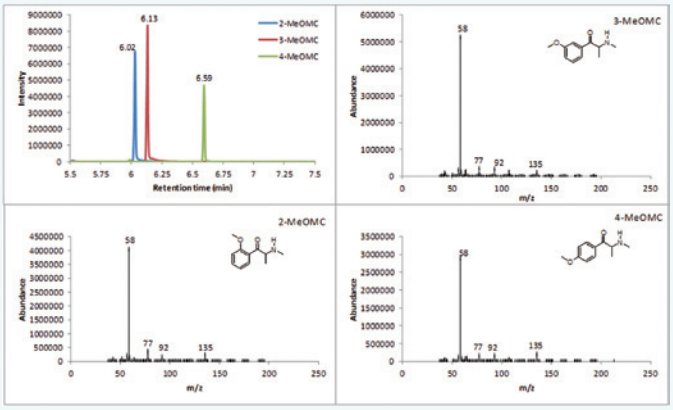

Figure 1: Gas chromatogram and mass spectra of 2-, 3-, and 4-methoxymethcathinones (MeOMC) using the routine in-house method.

Experimental Conditions

Reference Material

Commercial drug standards of 2-, 3-, and 4-FEC were purchased from Cayman Chemical with ≥ 98% purity. 1 mg of each drug standard was dissolved in 1 mL of HPLC grade methanol. A mixture solution of the three FEC isomers for GC/MS analysis was prepared by taking 100 µL of each drug standard (1 mg/mL in HPLC grade methanol) and placing them in a GC vial fitted with an insert. Solutions for LC analysis were prepared at 1 µg/mL of HPLC grade methanol.

Gas Chromatography

The 2-, 3-, and 4-FEC samples were analysed using an Agilent 6890N GC equipped with an Agilent 5973 quadrupole mass-selective detector with the following parameters for the routine in-house method:

Oven Temperature: 80°C (3 min) → 300°C (9 min) at 40°C / min
Solvent Delay: 3 min
Inlet Temperature / Pressure: 280°C / 8 psi
Run Time: 17.5 min
Injection volume: 1 µL
Split ratio: 40:1
Scan Range: 35 – 480 amu
Column: HP 5-MS (12.5 m × 0.25 mm × 0.25 µm)

To enhance the resolution of the three positional isomers, the following parameters were changed for the optimized method:

Oven Temperature: 100°C for 15 min
Run Time: 15 min
Injection volume: 0.1 µL

Liquid Chromatography

The FEC isomers were also analysed using a Shimadzu Nexera UHPLC coupled with an LCMS-8030 triple quadrupole mass spectrometer with the following parameters:

Mobile Phase: 5% Acetonitrile (0.1% formic acid): 95% H2O (0.1% formic acid)
Flow rate: 0.25 mL / min
Column: Phenomenex Kinetex 2.6µ Biphenyl 100A, 100 mm × 2.1 mm
Column temp: 30°C
Injection Volume: 1 µL

Results and Discussion

Figure 2 shows the gas chromatogram of 2-, 3-, and 4-FEC obtained using the routine in-house method. The separation of the three isomers was less than our laboratory criteria of minimum 0.1 minute between peaks and $R^s \geq 1.5$. In addition, the mass spectra of all three FEC isomers were too similar to facilitate a positive identification of the specific isomer through mass fragmentation pattern match (Figure 2). Different isothermal/gradient temperatures for the gas chromatography as well as injection volumes were investigated to enhance the separation of the three FEC isomers. The optimum conditions for the separation of these three isomers were achieved by injecting 0.1 µL of the mixture solution into the GC with a split ratio of 40:1 and holding the temperature at 100°C for 15 minutes (Figure 3). The 2-FEC isomer eluted at 8.42 minutes followed by 3-FEC and 4-FEC which eluted at 8.94 minutes and 9.23 minutes respectively. The Rs values were determined to be 3.4 for 2-FEC & 3-FEC and 1.6 for 3-FEC & 4-FEC. We also explored tandem MS-MS to differentiate these isomers, more specifically the multi reaction monitoring (MRM) experiment [6]. However, these experiments did not discriminate the FEC isomers from one another as similar transitions were obtained for all of them.

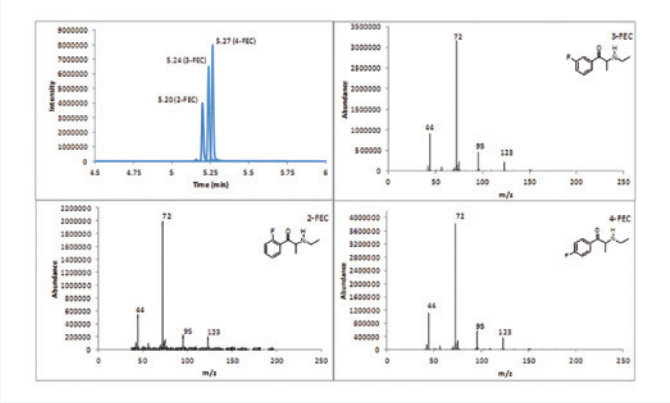

Figure 2: Gas chromatogram and mass spectra of 2-, 3-, and 4-fluoroethcathinone (FEC) using the routine in-house method.

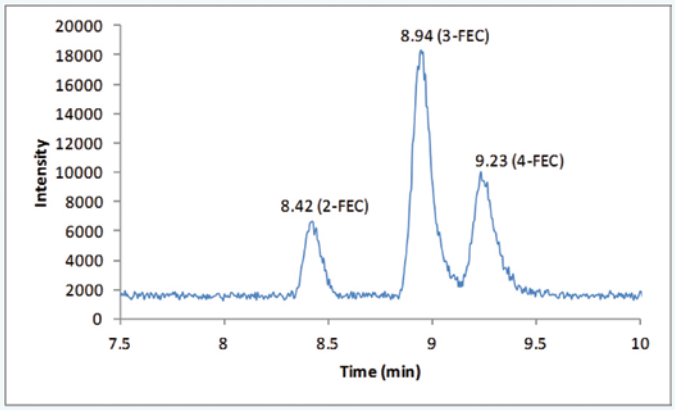

Figure 3: Gas chromatogram of the 2-, 3-, and 4-FEC obtained at the isothermal temperature of 100 °C.

The use of LC (coupled to a MS detector) was also utilised in the discrimination of the FEC isomers. An optimised mobile phase consisting of 5% acetonitrile (0.1% formic acid) and 95% de-ionised water (0.1% formic acid) using biphenyl column provided sufficient resolution of the FEC isomers, Rs = 1.5 (2-FEC & 3-FEC) and 2.7 (3-FEC & 4-FEC) (Figure 4). The method facilitated the elution of 2-FEC at 8.06 minutes followed by 3-FEC at 8.56 minutes and then by 4-FEC at 9.58 minutes. In addition, the use of C18, pentafluorophenyl and phenylhexyl columns were also explored but were ineffective in resolving the FEC isomers.

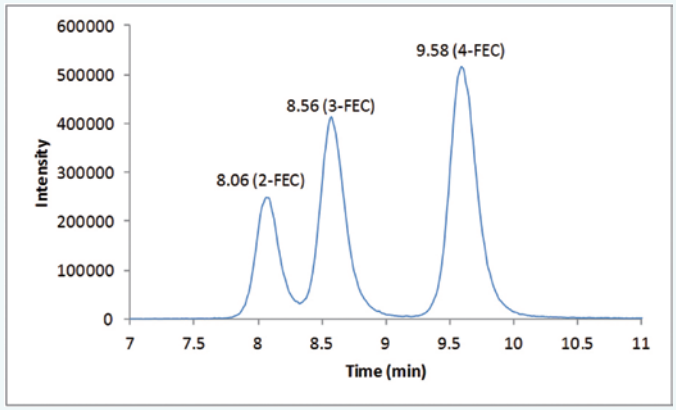

Figure 4: Liquid chromatogram of 2-, 3-, and 4-FEC.

The better performance of the biphenyl column as compared to other columns examined could be due to its ability in having a different π-π interaction with each of the FEC isomers. This interaction is absent in C18 column since it does not have a pi-character [7] and as a result, discrimination between FEC isomers was not observed. The phenylhexyl and pentafluorophenyl columns are expected to have π-π interaction with FEC isomers but are not as effective as the biphenyl column. Biphenyl column provides a larger electron cloud compared to single ring phenyl phases (pentafluorophenyl and phenylhexyl) resulting in high degree of aromatic selectivity and consequently, thereby providing a better separation of aromatic compounds [8].

Conclusion

Isomeric discrimination of 2-, 3-, and 4-FEC positional isomers was investigated via MRM transitions experiment and chromatographic separation. MRM transitions experiment failed to differentiate the isomers. However, isomeric separation of the three FEC isomers was achieved through liquid and gas chromatographies after optimisation. In LC, the use of biphenyl column as the stationary phase and 5% acetonitrile in water (0.1% formic acid) as the mobile phase facilitated the separation. Similarly, FEC isomers were separated by GC at the isothermal temperature of 100°C. To the best of our knowledge, this is the first reported example of LC and GC separations of the three FEC isomers. The chromatographic conditions determined in this study will be useful for other laboratories to achieve the separation of the three FEC isomers for identification.

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LC-MS/MS Determination of Oleandrin in Biological Samples

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Introduction

Nerium indicum Mill (*Nerium Oleander* L) is an evergreen shrub, which has a long history of cultivation in China and throughout the world. Oleander is regarded as one of the most toxic plants in the world, containing oleander glycosides and other toxic ingredients. The leaf, root, bark, flower and seeds of oleander are toxic, and the most abundant toxins are oleandrin [1,2] and nerine [3,4]. The chemical structure of oleandrin is shown in Figure 1.

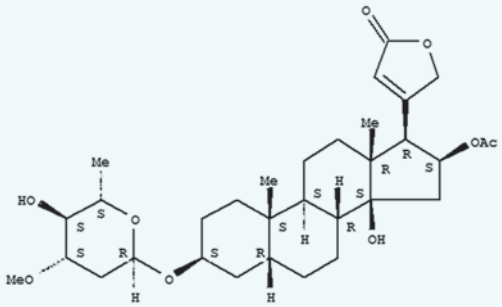

Figure 1: Chemical structure of oleandrin.

There are many reports of severe poisoning or death due to an overdose of oleander, mostly due to the patients themselves or their family members picking fresh oleander leaves and soaking them in water for consumption [5,6]. The domestic literature on oleander is mostly about the analysis of parts of the plant, case reports of poisoning and some reports regarding oleander poisoning being determined in human samples. In this paper, we present a G2-S QTof method with accurate mass determination for the identification of oleandrin in human samples.

Materials and Methods

Chemicals and standards

Chromatographic grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany), and oleandrin standard was purchased from Shanghai Jimian Industrial Co. Ltd. (Shanghai, China). Standard stock solutions of oleandrin at 1 mg/mL concentration were prepared using distilled water and stored at -20ºC. Working standards were subsequently prepared from the standard stock solutions by appropriate sequential dilutions with distilled water and stored at 4ºC. All the other chemicals used in the experiment were of analytical grade.

Extraction Procedure

For qualitative analysis, 4 mL of acetonitrile was added to 1 mL of blood. The mixture was shaken for 1 min with a vortex mixer and centrifuged (10,000 × g, 10 min, 4ºC). A 100 µL aliquot was used for the analysis. The extract was reconstituted in 0.5 ml 50:50 acetonitrile/water and analysed.

Instrumentation

The analysis of oleander was performed on a Waters Xevo G2-S QTof (Waters, USA). Separation was performed on an Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm) which was maintained at 35ºC. A gradient elution using 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B) at a flow rate of 0.4 mL/min, is as shown in Table 1. Total run time was 5.0 min. The injection volume was 2 µL.

Time	A%	B%
0	1	99
4.0	95	5
5.0	95	5
5.1	1	99
6.5	1	99

Table 1: HPLC Gradient Profile.

Mass spectra were obtained using a G2-S QTof system with electrospray ionisation (ESI). Parameters used were as follows: capillary voltage of 3.0 kV, desolvation gas of 1000 L/hr, cone gas of 50 L/hr, desolvation temperature of 550°C and ion source temperature of 120°C. Nitrogen, obtained from a nitrogen generator (99.93%), was used for the desolvation.

Results and Discussion

The calibration curves were linear in the range of 1-500 ng/mL for oleandrin, with correlation coefficients that were routinely greater than 0.996 in all cases. The detection limit of oleandrin was 0.1 ng/mL (S/N=3). To establish the accuracy of the calibration curve, spiked samples at 1 ng/mL, 50 ng/mL and 500 ng/mL of oleandrin were measured by the above method. The recoveries were 88.1% - 96.2%. The intra- and inter-day precisions were within 10.0% at the three concentrations.

This study established a m/z 577.328 for qualitative and quantitative analysis of oleandrin. The analysis time of this method is only 6.5 min. As the procedure proved to be sensitive, selective and reproducible, the method developed was applied to a fatal poisoning case. In this case, students were poisoned because of mistaking fresh oleander leaves as honeysuckle, and drank the tea made with it. Oleandrin was detected in their blood samples. Quantitative analysis was not done due to insufficient amount of the samples submitted.

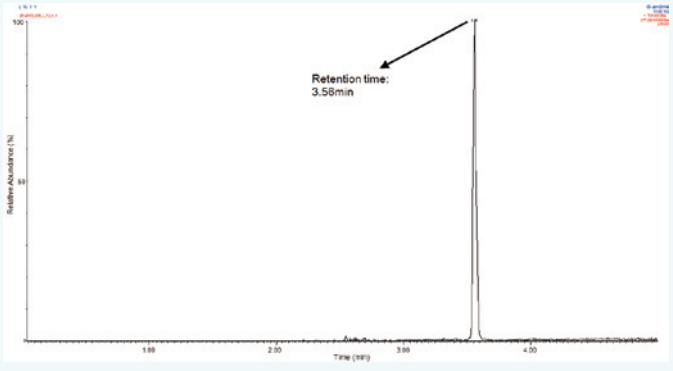

Figure 2: TIC spectrum of oleandrin in human blood.

Conclusion

The method is shown to be sensitive, accurate, rapid and suitable for forensic toxicology applications to test for oleandrin. Furthermore, the extraction procedure is simple, effective and suitable for the application to complex matrices like tissue samples.

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Rapid Determination of γ-hydroxybutyrate in Human Urine by LC-MS/MS Method

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Introduction

γ-hydroxybutyrate (GHB) is becoming one of the most popular recreational drugs. As a result of its strong sedative and amnesiac effects, GHB has been implicated in a number of drug-facilitated sexual assault (DFSA) cases. The natural presence of GHB in the human body and its rapid elimination after ingestion makes it difficult to detect and to evaluate its roles in suspected GHB-facilitated assaults. The chemical structure of γ-hydroxybutyrate is shown in Figure 1.

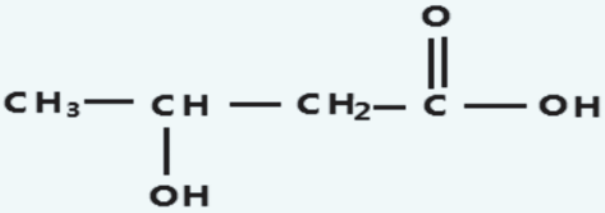

Figure 1: GHB chemical structure.

Methods of analysis of γ-hydroxybutyrate using rapid colourimetric screening test, GC-MS and HPLC-UV have had limited success, which does not give the information necessary to identify it and may not be sensitive enough to determine GHB [1-4]. We therefore designed a specific and sensitive method using liquid chromatography-tandem mass spectrometry (LC-MS/MS). However, there are few reports on the identification of γ-hydroxybutyrate in human samples. The aim of our work is to identify γ-hydroxybutyrate in the human urine in a DFSA case by LC-MS/MS.

Case history

In a case which happened on campus, three female students were stabbed by a strange man. The victims all had symptoms of dizziness, nausea and weakness. One of the victims was rushed to hospital and a urine sample was collected for toxicological analysis.

Materials and Methods

Chemicals and standards

Chromatographic grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany), and γ-hydroxybutyrate standard was purchased from Lancaster Synthesis (England). Standard stock solution containing 1 mg/mL of γ-hydroxybutyrate was prepared in distilled water and stored at -20°C. Working standards were prepared from standard stock solution by appropriate sequential dilutions with distilled water and stored at 4°C. All the other chemicals used in the experiments were of analytical grade.

Extraction Procedure

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 4 mL aliquot was used to clean-up by gel permeation chromatography. The extract was reconstituted in 0.5 mL 50:50 acetonitrile/water and analysed.

Instrumentation

The analysis of γ-hydroxybutyrate was performed on Waters Xevo TQ MS (Waters, USA). Separation was performed with an Acquity UPLC BEH C18 column, 2.1 × 50 mm, packed with 1.7 µm particles, which was maintained at 35°C. A gradient elution using water (with 0.1% formic acid of 10 mM ammonium acetate, mobile phase A) and acetonitrile (aqueous mobile phase B) at a flow rate of 0.5 mL/min was shown in Table 1. Total run time was 4.0 min.

Time	A%	B%
0	2	98
0.3	2	98
2.0	50	50
2.5	90	10
4.0	2	98

Table 1: Gradient Profile.

Mass spectra were obtained using a Acquity UPLC system with electrospray ionisation (ESI). The mass spectrometer was operated in multiple reaction monitoring (MRM) mode. Parameters were as follows: capillary voltage was set to 2.05 kV, desolvation gas to 500 L/Hr, cone gas to 50 L/Hr, desolvation temperature to 200°C and ion source temperature to 150°C. Nitrogen, obtained from a nitrogen generator (99.93%) was used for desolvation. MRM transitions of m/z 102.88→56.97 and 105.88→84.90 were monitored for γ-hydroxybutyrate at collision energy of 10 V and 15 V, respectively.

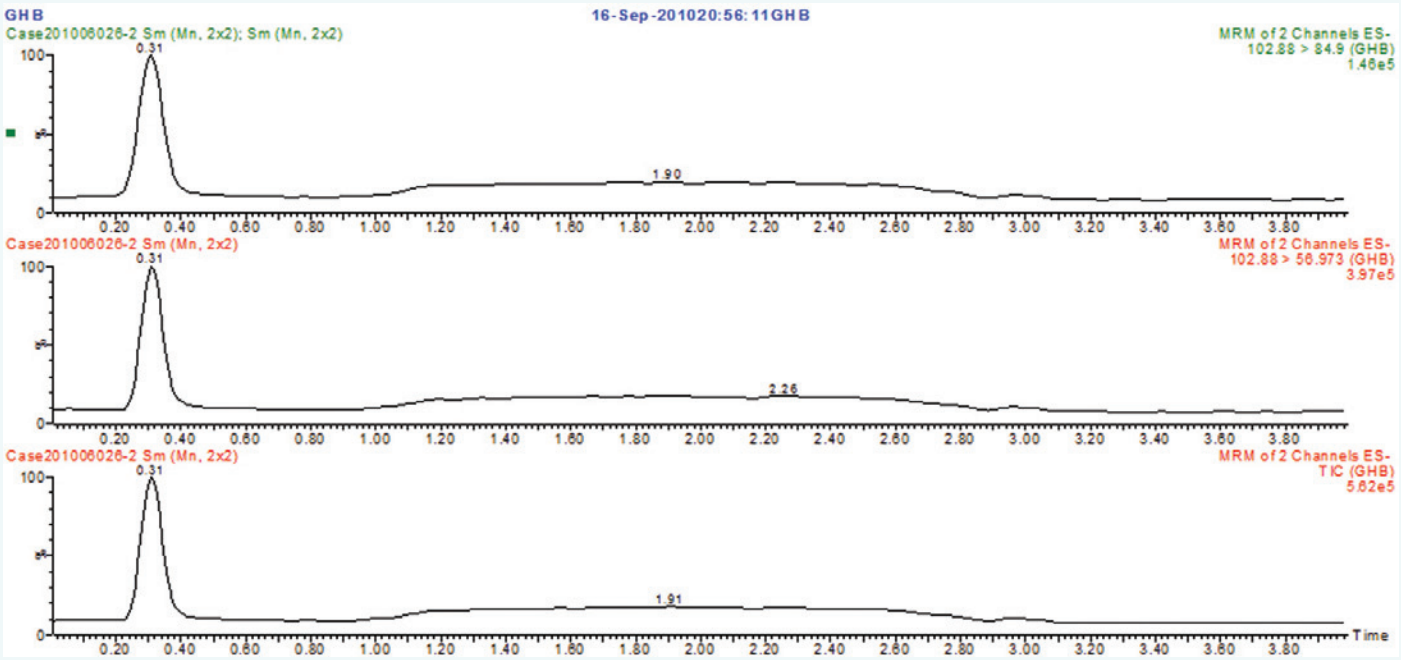

Figure 2: LC-MS/MS chromatograms with two MRM transitions monitored in the victim's urine sample of the case report.

Results and Discussion

The calibration curve was linear in the range of 0.5-100 µg/mL for γ-hydroxybutyrate, with correlation coefficients that were routinely greater than 0.998 in all cases. The detection limit of γ-hydroxybutyrate was 0.04 µg/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 γ-hydroxybutyrate and measured by the above method. The recovery was 86.2% - 98.6%. The intra- and inter-day precision was within 6.5% at three concentrations.

This study established a LC-MS/MS qualitative and quantitative analysis method for γ-hydroxybutyrate. The analysis time of this method is only 4.0 min. Compared with traditional methods, this method greatly shortens the analysis time and improves sensitivity. Since the procedure proved to be sensitive, selective and reproducible, the method developed was applied to the fatal case presented. In this case, the LC-MS/MS results showed the presence of γ-hydroxybutyrate in urine. The LC-MS/MS chromatograms obtained from the extract of the victim's urine are shown in Figure 2.

Conclusions

The UPLC-MS-MS method is shown to be appropriate for screening and identification of γ-hydroxybutyrate in biological specimens. The extraction procedure is simple and effective. The method is suitable for the application to complex matrices like tissue samples. The low limit of detection allows for the detection of γ-hydroxybutyrate after a long period of time upon poisoning.

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Elemental Profiling of Various Types of Glass Using LA-ICP-MS

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Abstract

This paper outlines the value of the laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) technique in forensic glass analysis. The elemental profile obtained by this technique can differentiate the different types of glass, provides excellent discriminating power in pair-wise comparisons of automotive glass and may classify some of the automotive glass by their manufacturing plants.

Introduction

Glass is a common type of trace evidence that can be found at crime scenes. Refractive Index (RI) has been traditionally used to discriminate glass samples. However, the range of variation for RI values has narrowed over time, likely due to better quality control of optical and physical properties during the manufacturing process, which reduces the discriminating power of RI.

To complement RI, elemental analysis is usually carried out to increase the evidential value of glass [1-4]. The trace elements present in glass vary due to:

- The components in the raw materials used, which is mainly sand, limestone and soda ash,
- Modifiers such as Na_2O, CaO and MgO which are intentionally added to lower the melting point and viscosity during the manufacturing process,
- Leeching from machinery and the environment during manufacturing.

The laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) technique has detection limits lower than that of scanning electron microscopy SEM-EDX and XRF. Its ability in profiling very trace amounts of elements provides greater discriminating power in forensic glass analysis [5-7].

Materials and Methods

Instrumentation, Parameters and Method

The system used for this study consists of an Agilent 7700x ICP-MS system and a CETAC LSX 213-G2 laser ablation system. The typical parameters used for our LA-ICP-MS combination are as shown in Table 1.

The element menu comprises the following elements and isotopes: ^{7}Li, ^{11}B, ^{23}Na, ^{24}Mg, ^{27}Al, ^{29}Si, ^{39}K, ^{42}Ca, ^{49}Ti, ^{55}Mn, ^{57}Fe, ^{85}Rb, ^{88}Sr, ^{90}Zr, ^{118}Sn, ^{137}Ba, ^{139}La, ^{140}Ce, ^{146}Nd, ^{178}Hf and ^{208}Pb. The mass spectra for the samples were collected as transient signals, with 30 seconds of gas blank, followed by 60 seconds of ablation, and 30 seconds of wash-out time.

The GLITTER software package was employed to process the transient signals. The NIST SRM 612 was used as a one-point external calibration and drift corrector. ^{29}Si was used as the internal standard.

Table 1: Typical parameters used for LA-ICP-MS analysis

Instrument	Parameter	Value
Laser Ablation	Ablation mode	Single spot
	Spot size	50 µm
	Laser power	80%
	Shot frequency	10 Hz
	Number of shots	600
	He carrier gas flow	0.80 L/min
ICP-MS	RF power	1350 W
	Ar carrier gas flow	0.50 L/min
	Sampling depth	4.5 mm
	Dwell time	10 ms
	Acquisition mode	TRA

Sample Collection

90 glass samples were collected and analysed. Of the 90 samples, 11 were architectural glass, 64 were automotive windscreen glass, 5 were automotive headlamp glass, and 10 were container glass.

The 64 windscreen glass samples were collected from 64 different windscreens. All the samples were collected from the exterior glass pane. The samples were from 4 different Asian makes, with 2 different manufacturing plants for each make. A summary of the breakdown of the analysed samples is shown in Figure 1. The cars from which the windscreens were obtained from were manufactured between 2003 and 2013 and were of various models, as shown in Table 2.

HON		
Model	Car Manufacture Year	Number of samples
CVC	2007	1
	2008	2
CRV	2007	1
	2008	1
	2010	1
	2012	1
JZZ	2005	2
	2006	1
	2008	1
ODS	2005	1
	2006	1
	2007	1
STM	2009	2

HYU		
Model	Car Manufacture Year	Number of samples
AVT	2007	1
	2008	1
	2009	1
I45	2012	1
MTR	2003	1
	2004	1
STF	2008	1
	2010	1
	2011	1
SRX	2011	1
TRJ	2005	3
TSN	2008	1
VLS	2012	2

NIS		
Model	Car Manufacture Year	Number of samples
MAR	2007	1
CBSR	2009	1
CFR	2006	3
GTR	Unknown	2
LAT	2005	1
	2008	1
SNY	2004	1
	2005	1
	2007	1
SLP	2006	1
	2008	2
TNA	2008	1

TOY		
Model	Car Manufacture Year	Number of samples
CMR	2006	1
	2009	1
	2012	1
ALT	2009	3
	2010	1
	2012	1
DYN	2010	1
	2012	1
	2013	3
PCN	2006	2
PVIA	2012	1

Table 2: Breakdown of samples by make, model and car manufacture year.

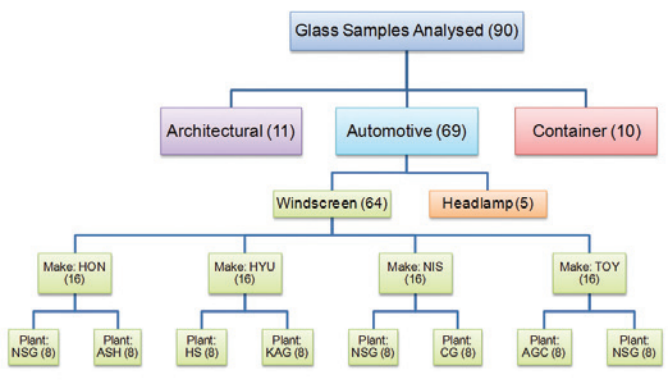

Figure 1: Breakdown of samples collected and analysed.

Results and Discussion

Differentiation of Glass Types

Headlamp glass is mainly borosilicate glass. The elemental composition of borosilicate glass is observed to be vastly different from that of container and float glass, with high concentrations of boron (B) and low concentrations of magnesium (Mg). A plot of the concentrations of Mg against concentrations of B is shown in Figure 2. The headlamp borosilicate glass samples can be easily differentiated from float and container glass.

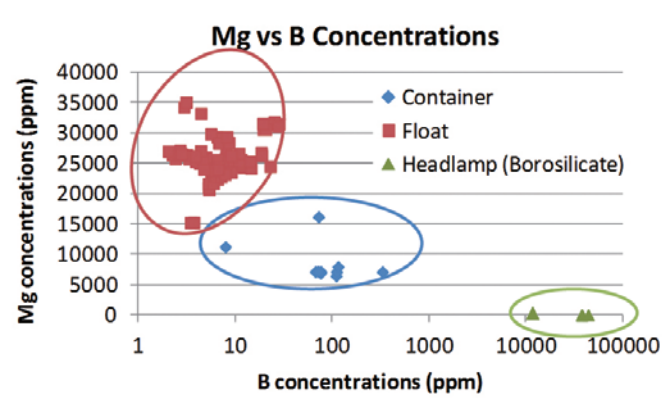

Figure 2: Plot of Mg against B concentrations.

Comparing between container and float glass, container glass tends to have higher Ca concentrations and lower Mg concentrations. Its Ca/Mg ratios are slightly higher and more variable than that of float glass. This is evident in the plot of Mg against Ca concentrations, as shown in Figure 3. In addition, container glass has more variable Pb concentrations than that of float glass, as shown in the plot of Pb concentrations against Ca/Mg ratios in Figure 4.

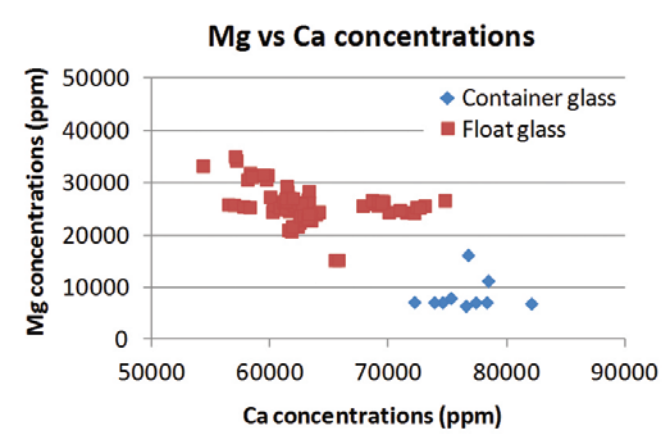

Figure 3: Plot of Mg against Ca concentrations of container and float glass samples.

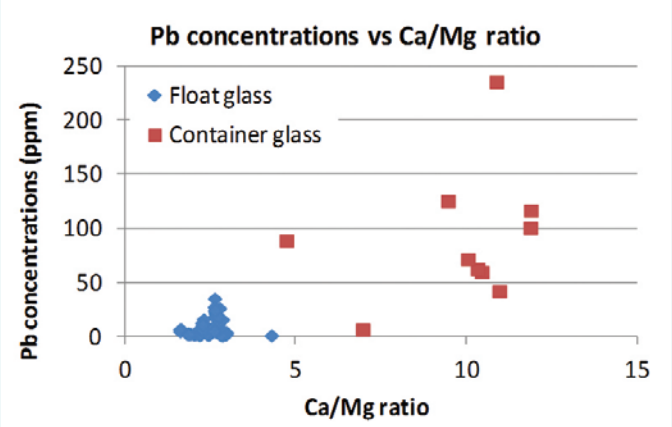

Figure 4: Plot of Pb concentration against Ca/Mg ratios of container and float glass samples.

Automotive Windscreen (Pair-wise Comparison)

Pair-wise comparison was carried out among the 64 windscreen samples analysed. The match criterion for the pair-wise comparison used is that the mean concentrations of analysed elements (^{7}Li, ^{23}Na, ^{24}Mg, ^{27}Al, ^{39}K, ^{42}Ca, ^{49}Ti, ^{55}Mn, ^{85}Rb, ^{88}Sr, ^{90}Zr, ^{137}Ba, ^{139}La, ^{140}Ce, ^{146}Nd, ^{178}Hf and ^{208}Pb) in the "question" sample will fall within 4 times the standard deviation, with minimum 3% relative standard deviation, of the "control" sample to render the "question" sample to be indistinguishable from the "control" sample.

With the 64 analysed windscreen glass samples, 2016 pair-wise comparisons were carried out. Of the 2016 pair-wise comparisons, 7 pairs (0.4%) were indistinguishable by LA-ICP-MS using the determined match criterion. Using the RI, 247 (12.3%) pairs were indistinguishable using a pre-determined match criterion. With a combination of LA-ICP-MS and RI, 6 pairs (0.3%) were indistinguishable. Thus, with the RI technique alone, the discriminating power is 87.7%. This can be improved to 99.7% with the use of LA-ICP-MS in conjunction with RI. Of the 6 indistinguishable pairs, each pair of sample was from the same manufacturing plant, with the same car manufacturing year. Thus, there is a possibility that the pair of sample could have been manufactured at around the same time in the same plant which resulted in a similar elemental profile.

Among the analysed elements, trace elements are more discriminating than major elements like Mg, K, Ca and Al in pair-wise comparisons. Of the trace elements, the most discriminating element is Ce (95% discriminating power), followed by Pb (91%) and Ba (90%). The concentration of Ce in the 64 samples has a very large variation, with a range of 1 ppm to 15000 ppm, which makes it highly discriminating among the analysed samples.

Automotive Windscreen (Classification)

The classification of windscreen glass by the vehicle make or manufacturing plant is useful in narrowing down a search for a particular vehicle, for example, providing an investigative lead in a hit-and-run accident. The classification can be done by observing distribution patterns in concentrations or ratios of certain elements and using Principle Component Analysis (PCA).

Of all the 8 manufacturing plants as shown in Figure 1, they are located in 3 different countries. HYU-KAG and HYU-HS are in the same country, TOY-AGC is in another country, and the rest of the manufacturing plants are in a third country.

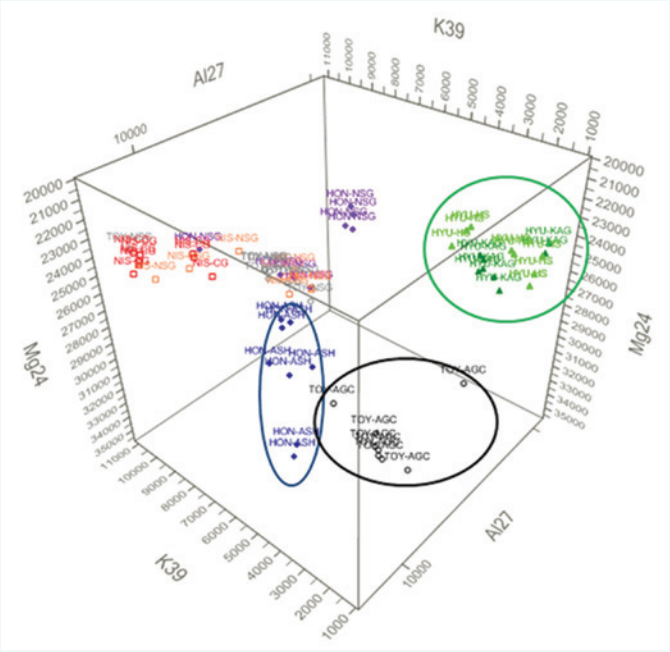

Figure 5: 3-dimensional plot of the concentrations of K, Mg and Al of windscreen samples.

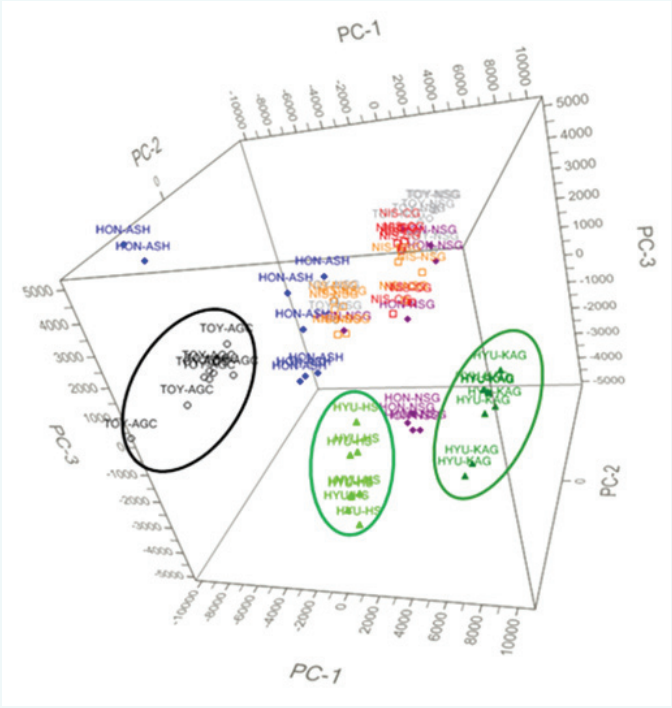

Figure 6: 3-dimensional PCA plot of windscreen samples.

From the 3-dimensional plot of the concentrations of K, Mg and Al (Figure 5), HYU-KAG and HYU-HS can be found in one distinct cluster, TOY-ASH in another cluster, and HON-ASH in a third distinct cluster. From the 3-dimensional PCA plot (Figure 6), HYU-KAG and HYU-HS can be separated into 2 clusters and TOY-ASH is in another cluster.

Based on these two plots, there is distinct grouping by manufacturing plants from different countries but samples from manufacturing plants in the same country are more difficult to classify. Unlike pair-wise comparisons, major elements like K, Al, Mg and Ca are more important for classification than trace elements.

Conclusion

The LA-ICP-MS is a useful technique for glass analysis. Based on the elemental profile, different types of glass such as headlamp, container and float glass can be differentiated. It has excellent discriminating power, with 99.6% of pair-wise comparisons being distinguishable. Based on the elemental profile of major elements, some of the automotive windscreen samples can be classified according to manufacturing plants.

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A Case of Processing Videos by VIS

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Introduction

Video data, which is a kind of intuitive physical evidence, plays an important role in some cases. However in many cases, low-quality videos can be due to low resolution of video surveillance, occurring time of cases (which are always at night), dust and webs covering cameras because of poor maintenance, motion blur caused by high speed movement of target, defocus blur because of poor focusing control, *etc* [1]. It is thus impossible to recognise suspects or suspicious vehicles directly and clearly. These problems can be resolved by techniques like image processing and image restoration to improve the image quality for obtaining clues. This paper introduces a successful case of processing the video of a license plate by VIS.

Background Information

In a case which happened in Hebei Province this year, a laptop computer and other items were stolen from the victim's car. The suspect fled in a van, whose license plate was captured by surveillance cameras. The inspection unit requested for the processing of the videos to obtain the license plate number of the van.

Materials and Methods

Instrumentation

In this paper we used the VIS software, which has a main function of providing an overall analysis environment for videos, sequence images and is able to comprehensively analyse videos and images in a single frame. Through means of preprocessing, conventional and advanced processing, it can perform various tasks including shape correction, image enhancement, noise reduction, de-blurring, image restoration and super resolution enhancement in notable areas to help find key evidence against criminals [2].

Analysis

After playing the video with Baofeng player, we ascertained that the format of the video was "rmvb", the size of the file was 52.4M, the single frame image size was 1280×960 and that it was a high definition video. However, the license plate number of the target van appeared fuzzy and was hard to recognise.

Workflow

The workflow of video image processing of such cases using VIS Software is shown in Figure 1. However it is worth noting that not all steps will be used and some steps will also be reversed to facilitate processing.

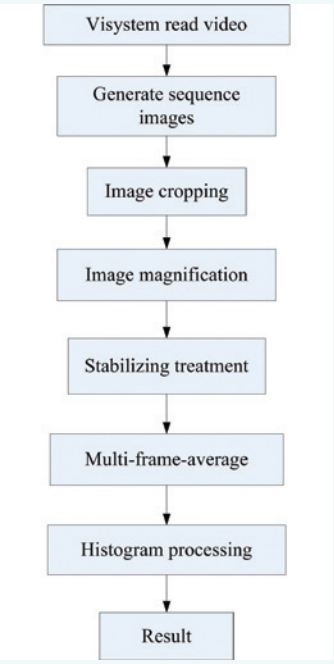

Figure 1: The workflow of video processing in VIS software.

Processing Procedure

We used VIS system to process the video image. The main steps are as follows:

(1) Using VIS software, we transformed the video into sequence images, repeatedly watched each frame of the video, and selected six high-quality images, of which the sequence is shown in Figure 2. This step would affect the final result.

(2) The sequence was stabilised using optic flow method, and the result, as shown in Figure 3, was averaged to reduce the noise.

(3) In order to reduce the operation quantity, we cropped Figure 3 to get the license plate area as shown in Figure 4.

(4) We did motion de-blurring to Figure 4. After continuous attempts, we knew that the motion angle was 27°. The parameter settings are as follows: Angle: 27°; Motion radius: 12.8; Noise: 20. We saved the image to Figure 5. This step was very important and we had to adjust the parameter more than once until we obtained a good result.

(5) Figure 6 was obtained from histogram processing of Figure 5.

Figure 2: Sequence Images.

Figure 3: The image after stabilizing and multi-frame averaging.

Figure 4: Cropping the Figure 3.

Figure 5: Motion De-blurring.

Figure 6: Histogram processing

Results and Discussion

In Figure 6, the last four digits of the license plate could be recognised as either “6567” or “5567”. The first 2 digits of the license plate could be deduced through the location of the surveillance camera. Based on the model and color of the van, we made a fuzzy search in the motor vehicle information database to determine the van in question and find clues to identify the criminal.

Conclusion

With Visystem, we could process the image of target license plate number. Thus, it can provide a solid foundation and effective evidence for some cases even by getting only 1 digit of the license plate number.

References

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2.	Department of Scientific Services, Brunei Darussalam	22.	Forensic Science Center of Guangdong Provincial Public Security Department, People's Republic of China
3.	Centre for DNA Fingerprinting and Diagnostics (CDFD), India	23.	Guangzhou Forensic Science Institute, People's Republic of China
4.	Department of Police Medicine of the Indonesian National Police, Indonesia	24.	Institute of Forensic Science, Ministry of Public Security, People's Republic of China
5.	Eijkman Institute for Molecule Biology, Indonesia	25.	Institute of Forensic Science, Tianjin Public Security Bureau, People's Republic of China
6.	Forensic Laboratory Centre of Indonesian National Police Headquarters, Indonesia	26.	The Institute of Evidence Law and Forensic Science, China University of Political Science and Law, People's Republic of China
7.	Indonesian Association of Forensic Pathologist, Indonesia	27.	Laboratory Service, Philippines Drug Enforcement Agency, Philippines
8.	Laboratory of National Narcotics Board, Indonesia	28.	National Bureau of Investigation, Philippines
9.	Coast Guard Research Institute, Korea	29.	Natural Sciences Research Institute, University of the Philippines Diliman Quezon City, Philippines
10.	Graduate School of Forensic Science, Soon Chun Hyang University (GFSCH), Korea	30.	Philippine National Police Crime Laboratory
11.	National Digital Forensic Centre (NDFC) of Supreme Prosecutor's Office, Korea	31.	Health Sciences Authority, Singapore
12.	National Forensic Service, Korea	32.	Central Institute of Forensic Science, Thailand
13.	Scientific Investigation Center of Korean National Policy Agency, Korea	33.	Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University, Thailand
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15.	Food and Drug Quality Control Center, Lao PDR	35.	Department of Medical Sciences, Thailand
16.	Forensic Science Department of Judiciary Police, Macau SAR	36.	Faculty of Medicine, Chiang Mai University, Thailand
17.	CyberSecurity, Malaysia	37.	Human Genetics Unit, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Thailand
18.	Department of Chemistry, Malaysia	38.	Institute of Forensic Medicine, Police General Hospital, The Royal Thai Police, Thailand
19.	Royal Malaysia Police Forensic Laboratory (RMP Forensic Lab), Malaysia	39.	Office of Narcotics Control Board, Thailand
20.	Mongolian National Institute of Forensic Science, Mongolia	40.	Vietnam Forensic Science Institute, Vietnam

(As at 01 October 2014)

Date	Venue
16 Feb - 21 Feb 2015	American Academy of Forensic Sciences (AAFS) 67th Annual Scientific Meeting. Orlando, Florida, USA.
2 Aug - 8 Aug 2015	International Association for Identification (IAI) Annual International Educational Conference. Sacramento, California, USA.
8 Aug - 13 Aug 2015	American Society of Questioned Document Examiners (ASQDE) 73rd Annual General Meeting. Toronto, Canada.
30 Aug - 4 Sept 2015	The International Association of Forensic Toxicologists (TIAFT) 53rd Annual Meeting. Florence, Italy.
31 Aug - 5 Sept 2015	International Society for Forensic Genetics (ISFG) 26th International Congress. Kraków, Poland.
6 Sep - 11 Sep 2015	7th European Academy of Forensic Science (EAFS) Prague, Czech Republic
12 Oct - 15 Oct 2015	26th International Symposium on Human Identification (ISHI). Grapevine, Texas, USA.
18 Oct - 23 Oct 2015	Society of Forensic Toxicologists (SOFT) Annual Meeting, Atlanta, Georgia, USA.
TBA	Asian Forensic Sciences Network (AFSN) 7th Annual Meeting and Symposium, Beijing, PRC