ForensicAsia

THE ASIAN FORENSIC SCIENCES NETWORK NEWSLETTER | ISSUE 4 | 2012

AFSN President's Address

It seemed a short time ago when we last met for the 3rd Annual Meeting in Seoul, South Korea, and soon we will be looking forward to the 4th AFSN Symposium and workgroups/committee scientific session/workshop on 25 – 27 November and Annual Meeting on 28 November 2012 in Bangkok, Thailand. This international scientific event is an excellent forum for all the delegates and members to exchange new ideas and experiences and, at the same time, renew and strengthen our networking and collaborations.

On behalf of the Board Members, I would like to take this opportunity to express our sincere thanks and appreciation to Dr Chung Heesun, Director General of National Forensic Sciences (NFS), Korea, for her active participation and contributions to AFSN during her tenure as the International Liaison Officer (2009 – 2011) and as Vice-President (2011 – 2012) of AFSN, as we have been informed that she has left NFS. It has been a privilege to have the opportunity to know Dr Chung and to work with her. Her dedication and leadership have contributed tremendously towards the success and recognition of AFSN. She will definitely continue to play an active role in the field of forensic sciences as she is the President of the International Association of Forensic Science (IAFS) and President-elect for The International Association of Forensic Toxicologists (TIAFT). We wish her all the best and success in her new position. On behalf of all AFSN members, we would like to congratulate Dr Joongseok Seo, the new Director General of NFS, and welcome him as the Vice-president of AFSN.

I am pleased to inform that, as requested by the organiser, European Network of Forensic Science Institutes (ENFSI) of 6th European Academy of Forensic Science (EAFS) Triennial Conference 2012, 20 – 24 August, The Hague, Netherlands, all partner networks of the International Forensic Strategic Alliance (IFSA) have submitted video messages from their respective network's president/chairperson to be displayed at the EAFS 2012 Opening Ceremony. In addition, Dr Angeline Yap of the Health Sciences Authority, Singapore, Chairperson of the AFSN Illicit Drugs Workgroup, has presented on the activities and achievements on behalf of the AFSN Board during the IFSA workshop held during the conference.

This year, the IFSA Annual Meeting was held in Hobart, Tasmania, Australia, in conjunction with the 21st International Symposium on Forensic Sciences, 23 – 27 September 2012, organised by the Australian New Zealand Forensic Science Society (ANZFSS). Dr Lam Kian Ming (International Liaison Officer) and I represented AFSN during the Annual Meeting.

On invitation from Dr Justice Tettey, United Nations Office on Drugs and Crime (UNODC), IFSA held a special meeting in Vienna, Austria, on 28 – 29 February 2012. During the meeting, a Memorandum of Understanding was signed between six Regional Networks of Operating Forensic Laboratories, namely, American Society of Crime Laboratory Directors (ASCLD), ENFSI, Academia Iberoamericana De Criminalistica Y Estudios Forenses (AICEF), Senior Managers of Australian and New Zealand Forensic Laboratories (SMANZFL), AFSN, and the Southern Africa Regional Forensic Science Network (SARFS). Thus, with this international multilateral partnership together with continued commitment by member institutes to actively support and contribute to the activities that are being carried in various network workgroups/committee, more forensic science institutes in Asia will realise the benefits and importance of joining AFSN which will then further strengthen the forensic science community in Asia. So far, the AFSN secretariat has received seven new applications for membership, two from Thailand, and one each from People's Republic of China, Philippines, Bangladesh, India and Macau.

I look forward to seeing all of you in Bangkok, Thailand, in November 2012.

Mr Lim Kong Boon
AFSN President

Upcoming Events

Date	Event
18 Feb – 23 Feb 2013	American Academy of Forensic Sciences (AAFS) 65th Annual Scientific Meeting. Washington DC, USA.
4 Aug – 10 Aug 2013	International Association for Identification (IAI) Annual International Educational Conference. Providence, Rhode Island, USA.
24 Aug – 29 Aug 2013	American Society of Questioned Document Examiners (ASQDE) 71st Annual General Meeting. Indianapolis, Indiana, USA.
2 Sep – 6 Sep 2013	The International Association of Forensic Toxicologists (TIAFT) 51st Annual Meeting. Funchal – Madeira, Portugal.
2 Sep – 7 Sep 2013	25th International Society for Forensic Genetics (ISFG) Congress. Melbourne, Australia.
October 2013	24th International Symposium on Human Identification (ISHI). USA.
27 Oct – 1 Nov 2013	Society of Forensic Toxicologists (SOFT) Annual Meeting. Orlando, Florida, USA.
Oct / Nov 2013	AFSN 5th Annual Meeting and Symposium. Singapore.

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Editor's Address

Dear readers,

Welcome to Issue 4 of *ForensicAsia*! For this issue, we have a few changes to the Editorial Committee: We would like to thank Dr Chung Heesun (Editorial Advisor), Dr Kraisorn Ammawat (Guest Editor) and Ms Belinda Chiam (Editorial Assistant) for their contribution to the past issues of the newsletter; and extend our warm welcome to Dr Joongseok Seo (Editorial Advisor), Dr Christopher Syn (Deputy Editor), Dr Triyarith Temahivong (Guest Editor) and Ms Pauline Wong (Editorial Assistant). Many thanks to all the Editorial Committee Members for taking time out of your busy schedule to assist in reviewing the submitted articles.

There are many good and interesting articles submitted for this issue, with wide range of topics covering handwriting examination, drugs, paint, DNA, explosives, fibre, toxicology and clandestine laboratory investigations. In addition, there is a special invited article on "Challenges to Crime Scene Investigations – The CSI Effect" by Mr Joseph Blozis that traces the origin of this effect and how it is affecting crime scene investigation and court proceedings.

I hope that this issue will be an interesting read for all of you and I look forward to receiving more articles for the next issue!

Dr Angeline Yap
Editor

Editorial Committee

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

Updates of International Forensic Strategic Alliance Activities in 2012

Dr Angeline Yap, Dr Lam Kian Ming
AFSN

A Special Two-Day Meeting in Vienna (28-29 Feb)

The United Nations Office on Drugs and Crime (UNODC) hosted a special 2-day IFSA meeting on 28 to 29 February in Vienna, Austria, with a focus to strengthen international cooperation in the forensic sciences and create opportunities for strategic collaborations across the global forensic science community. Representatives of the six regional networks (ASCLD, AICEF, SMANZFL, ENFSI, AFSN and SARFS), together with UNODC representatives, were present at the meeting. Many important topics were discussed, including the setup of a dedicated IFSA website, the establishment of an Executive Director / Secretariat to support IFSA work, the importance of collaboration within the scientific working groups, the development of minimum standard documents, and training programmes to assist laboratories to build capabilities. At the end of the meeting, it was agreed that minimum standard documents, which guarantee acceptable level of quality and interoperability, be developed for the following 3 areas: Crime scene investigation, Drug analysis, and DNA.

IFSA representatives from regional forensic science networks at the Vienna Meeting.

IFSA has its Sixth Member!

The IFSA family has expanded from five to six to include the Southern Africa Regional Forensic Science Network.

The First IFSA Workshop at EAFS at The Hague (21 August)

IFSA held its first ever workshop on 21 August during the 6th European Academy of Forensic Science (EAFS) conference which was held in The Hague, Netherlands, from 20 to 24 August. The theme of the workshop was “Promoting International Cooperation in the Forensic Science Field - The role of regional networks of forensic scientists”. Representatives of the five regional networks (ASCLD, AICEF, SMANZFL, ENFSI and AFSN) presented the evolutions of the networks, the collaboration and cooperation of members within each network and with the wider forensic community, as well as the current and future focus of the networks.

The Annual IFSA Meeting at Hobart (23 September)

Representatives of the five regional networks (ASCLD, SMANZFL, ENFSI, AFSN and SARFS) met on the side of the ANZFSS on 23 September 2012.

A key highlight of the meeting was the discussion on the minimum standard documents that IFSA has been working on. These documents are aimed to provide guidance for developing forensic laboratories in the different forensic disciplines. At this meeting, the framework for these minimum standard documents was agreed, which would cover the following areas, contributing to the overarching theme of quality management:

1. Competence of personnel
2. Equipment and consumables
3. Collection, analysis, interpretation and reporting
4. Procedures, protocols and validation

Apart from the topics of Crime scene investigation, Drug analysis and DNA which are currently being worked on, minimum standard documents on another three new topics of Document Examination, Electronic Evidence and Latent Prints will be developed.

The other important development at this meeting is the agreement on the need for IFSA to have strategic objectives to set clear direction for its activities. The 5 areas identified are:

1. Emerging technologies and innovation
2. Minimum standard documents
3. Delivery/maintenance of IFSA website and public relations
4. Service delivery models - managing demands for forensic analysis
5. Scanning for research and development trends

It was a day of fruitful discussions with many exciting new ideas and projects for IFSA members to pursue, which will contribute to bringing IFSA to new heights.

The IFSA family at the Annual Meeting in Hobart.

Driving Under the Influence of Alcohol, Drugs and Psychoactive Medicines; the Results of the DRUID Project

Prof Dr Alain Gaston Verstraete
Ghent University, Belgium

An ambitious goal of the European Commission was to halve the number of road deaths between 2003 and 2010 [1]. To meet this goal, the European Commission launched the 5-year DRiving Under the Influence of Drugs, alcohol and medicines (DRUID) project in October 2006 in which 37 partners from 18 countries were involved. DRUID is an integrated project that studied the prevalence of psychoactive substances in drivers, the risk of being fatally injured or seriously injured in a car crash and the classification of medicines.

Prevalence of psychoactive substances among drivers

Thirteen countries (Belgium, Czech Republic, Denmark, Finland, Hungary, Italy, Lithuania, Norway, Poland, Portugal, Spain, Sweden and the Netherlands) participated in the DRUID roadside survey (2007-2009), and almost 50000 drivers were sampled. The prevalence of drivers who tested positive for at least one psychoactive substances ranged between 2.3% and 15.0%. Alcohol was the most frequently detected substance. The estimated EU mean prevalence for single alcohol use was 3.48% (≥0.1g/l), 1.49% (≥0.5g/l) and 0.4% (≥1.2g/l). The estimated EU mean prevalence for one or more illicit substances was 1.90% (range 0.22-8.20%) and for one or more psychoactive medicine 1.36% (range 0.17-2.99%). The EU mean prevalence for THC was 1.32% (range 0-5.99%). For cocaine, amphetamines and illicit opiates, the EU mean was below 1%. The estimated mean for use of a combination of different drug classes was 0.37%.

The DRUID hospital study sampled approximately 2500 seriously injured drivers (of car and vans) in 6 countries (Belgium, Denmark, Finland, Italy, Lithuania and the Netherlands) between 2007 and 2010 and 1118 killed drivers (of car and vans) in 4 countries (Finland, Norway, Portugal and Sweden) between 2006 and 2009. The percentage of injured drivers positive for at least one psychoactive substance ranged between 28 and 53% and it was between 31 and 48% among the killed drivers. Approximately 90% and 87% of the alcohol positive injured and killed drivers had a blood alcohol concentration (BAC) ≥0.5 g/l. Benzodiazepines were the most common medicinal drugs among both injured and killed drivers (0.0-10.2% and 1.8-13.3% respectively). Cannabis (0.5-7.6%) was the most common illicit drug in injured drivers and amphetamines (0-7.4%) in the killed drivers (amphetamines are popular in Scandinavia). Alcohol was found in combination with drugs in 2.3-13.2% of the injured drivers and in 4.3-7.9% of the killed drivers. Drug combinations were found in 0.5-4.3% and 0.4-7.3% of the injured and killed drivers respectively.

The risk of being fatally injured or seriously injured in a car crash

The results of the roadside surveys and the hospital studies were used in a case-control study which resulted in calculations of odds ratios of being seriously/fatally injured in a car crash while positive for psychoactive substances (Table 1). The highest odds ratios (20-200) were observed for BAC >1.2g/l and combinations of alcohol and drugs, while the lowest odds ratios (1-3) were observed for cannabis and a BAC between 0.1 and 0.5 g/l.

Classification of driving-impairing medicines

Driving a motor vehicle is a multifaceted task and it requires appropriate cognitive and psychomotor skills (e.g. alertness, concentration, reaction time). Psychoactive medications can adversely affect these driving-related skills, and, consequently, be a hazard to traffic safety [2, 3]. In the DRUID project, a European classification system of relevant therapeutic groups was developed for a total of 3054 medicines [4]. According to this classification a medicine can be categorised as category 0 (no or negligible influence on fitness to drive), category I (minor influence), category II (moderate influence) and category III (severe influence). Moreover, warning symbols or pictograms to inform patients were developed based on these four categories. In this new pictogram the various possible risks of impairing driving ability are displayed, horizontally, in a bar. From left to right, categories range from 0 to 3 and a different colour was attributed to each category. A traffic-light colour approach was followed, as people tend to associate the colour red to danger, the yellow to caution, and the green to safety (Figure 1).

Risk level	Risk	Substance group
Slightly increased risk	1-3	BAC 0.1-0.49g/l Cannabis
Medium increased risk	2-10	BAC 0.5-0. 9g/l Benzoylecgonine Cocaine Illicit opiates Benzodiazepines, Zolpidem or Zopiclone Medicinal opioids
Highly increased risk	5-30	BAC 0.8-1.19g/l Amphetamines Multiple drugs
Extremely increased risk	20-200	BAC ≥1.2 g/l Alcohol in combination with drugs

Table 1: Estimated relative risk of getting seriously injured or killed for various substance groups based on a case-control study performed in DRUID.

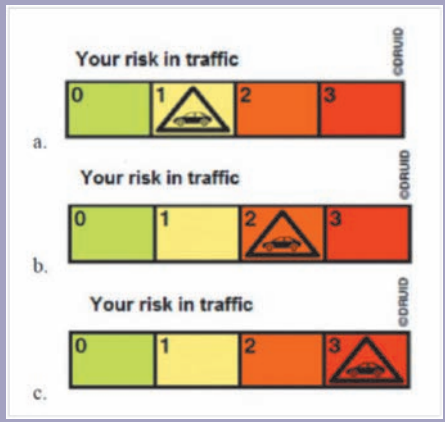

Figure 1: Pictogram for the categorisation of medicines according to their impairing effects on driving developed in the DRUID project: a) category 1, b) category 2, c) category 3.

Conclusion

The DRUID investigations showed that alcohol-impaired driving is the biggest problem in the European Union. The prevalence distribution of other psychoactive substances than alcohol showed more national variability. Based on the case-control study, the relative risk of serious injury or fatality is highly increased for alcohol (≥1.2g/l) and for the combination of different substances. Finally, the DRUID categorisation is a tool to improve prescribing and dispensing procedures both at a national and European level, and, therefore, as an instrument to better inform healthcare professionals and patients. All the DRUID results can be found at www.druid-project.eu.

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Challenges to the Crime Scene Investigator – The CSI Effect

Mr Joseph Blozis

The phrase Forensics means "seek the truth" and that is one of the most important goals for a crime scene investigator. In high school, there was the Forensic Club, they didn't recover finger prints, but debated, trying to determine the truth. As recently as in the mid 1990's, forensic science had little impact with crime scene investigations. Fingerprints were the primary means of identifying a suspect or suspects. Biological evidence such as body fluids were merely identified and typed. The best that hair evidence could provide was "consistent with" or "not consistent with". DNA was in its infancy stages and primarily used in major crimes such as homicides and sexual assaults. Analysis of the submitted samples was generally done only after an arrest was made or there was a known suspect.

Through the years, forensic sciences has evolved into an area of study that has incorporated its way into universities, public schools, private industry, law enforcement, the judicial system, and television. Not that long ago most people have never heard of forensic science, fingerprints, or DNA. Now, thanks to television, children and young adults throughout the world want to become CSI Investigators or Forensic Scientists. Law enforcement now uses a variety of chemicals and alternate light sources to aid in the recovery of trace evidence. How did this phenomenon occur? This question can be answered by answering the following question. What is one of the most popular drama series on television today and for the previous twelve years? You're correct, CSI.

The television show Crime Scene Investigation, also known as CSI Las Vegas debuted in October 2000 and remains on television today because of its huge success. Along with CSI Las Vegas, two other spin-off shows have been added. CSI Miami began in September 2002 and concluded in April 2012. CSI NY started in September 2004 and remains an ongoing program. Through the years CSI has won numerous television awards such as the Top TV Series among many others.

Besides the CSI shows, other shows such as Law and Order, Special Victims Unit, American Justice, Forensic Files, Cold Case Files, NCIS, all came into fruition. All these shows have enabled tens of millions of people worldwide to view and enjoy them. Viewers enjoy watching these shows because they find them extremely interesting and challenging. They love to solve the crime in order to find out "who did it" or "who didn't do it".

However, besides entertainment these shows have presented the criminal justice system with many challenges. The challenge is to provide jurors with as much forensic evidence as possible and when this cannot be accomplished, to explain to the jurors in detail the reasons why not. The onus is then put on the crime scene investigator to meet the challenge when processing a crime scene and when testifying about the crime scene.

Through the power of television these shows have not only entertained millions of people but changed their overall perspective of forensics and criminal investigations. Millions of people have become aware of the importance of forensics and the impact it has on the criminal justice system. With these changes come advantages and disadvantages.

One advantage of these shows is that they present a common trend for new careers within the field of forensic sciences. The most important aspect in a forensic investigation is not only to be able to identify the offender or offenders but is to exonerate the innocent. Unfortunately, there are individuals who are wrongfully incarcerated for crimes that they did not commit. Forensics can right this wrong.

Another advantage is that law enforcement agencies have learned and conformed to modern forensic technology. Law enforcement personnel watch CSI and other crime shows as well and want to implement the forensics they see into their investigations and casework.

Since 1990, over 1,588 episodes of CSI and Law and Order have been aired worldwide. Include the other crime drama shows and there are thousands of episodes. The majority of jurors are familiar with these shows, some more so than others. The probability of all the jurors to have never seen one or more of these shows is remote.

Some disadvantages that are to be considered are that some jurors may want more forensic evidence than is readily available. Others may exhibit confidence in their knowledge of forensic sciences and refuse to acknowledge what a true expert may provide in his or her testimony. Any doubt is grounds for acquittal. Reasonable doubt can be unreasonably altered. Another disadvantage is that law enforcement may be compelled to recover more items from a crime scene than otherwise needed resulting in a burden for their laboratory.

The viewers subsequently learn from these shows and believe what they see to be factual. The shows themselves are generally somewhat scientifically accurate, however, in reality the time it takes from processing and collection to come to a scientific conclusion is unrealistic. At times there are three or four simultaneous investigations going on and all four are solved within forty minutes including commercials. Every show has forensic results that impact the investigation. In reality, not every crime scene yields probative evidence. Sometimes there is no forensic evidence at all recovered from at a crime scene.

Many of these viewers are lay people who work "regular jobs" or raise families. One of their civic duties they may be eventually summoned to do is to become a juror on a criminal case. Once selected, they will have the important task to determine one's innocence or guilt. Their decision may literally result in a life or death sentence for the defendant on trial.

There have been numerous studies performed to determine whether or not there is a CSI effect on the jurors. Some studies such as the 2005 Arizona's Maricopa County Attorney's Office's Study concluded that these shows had a significant influence on jurors. The National District Attorney's Association has recognized an intense concern for the CSI effect.

Conversely, other studies such as the 2010 University of Wisconsin - Milwaukee Study, have concluded that crime shows had no effect on juror decisions.

In a famous 2011 Florida case, Casey Anthony, was acquitted of murdering her two year old daughter, Caylee. It was a circumstantial case and the jurors' forensic expectations were extremely high and the prosecution could not satisfy their expectations. The lack of forensic evidence had lead to Ms. Anthony's acquittal.

In another newsworthy 2004 California case, Robert Blake, an actor was acquitted for murdering his wife. The prosecutor, Steve

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Institute of Forensic Science, Ministry of Public Security, China

Dr Huang Xing, Dr Sun Jing, Dr Fu Huanzhang
Institute of Forensic Science, Ministry of Public Security, China

History and Background

The Institute of Forensic Science (IFS) was established in 1972 under the Ministry of Public Security (MPS), People's Republic of China. Its key mission is to provide scientific information for solving cases to public security organisations in all provinces, autonomous regions and municipalities directly under the Central Government through scientific analysis of physical evidence or on-site investigation. The IFS also provides intelligence for cases based on databases.

Organisation

The IFS comprises 30 subdivisions and is staffed with 303 persons, including 1 member of the Chinese Academy of Engineering, 121 senior scientific researchers and 109 mid-level scientific researchers, at the end of 2011. Among them, 32 persons hold PhD degrees, 71 persons hold Master degrees and 132 persons hold Bachelor degrees.

Case

The IFS consistently makes an effort to provide effective and precise results for meeting the requirements of the investigators and scientists in the Chinese forensic laboratory. The IFS undertakes the investigation of crime scenes and examination of evidences in major and complicated cases across the whole country and handles about 10,000 cases annually. These cases cover a wide range of fields including Forensic Pathology, DNA, Toxicological Analysis, Firearms, Explosives, Marks, Trace Evidence, Fingerprint Examination, Document Examination, Writing and Printing Materials Examination, Audio and Video Examination, and Computer Forensics. The IFS also deals with international cases and incidences related to forensic medicine, DNA, tool marks, explosive, trace evidence and toxicology etc.

The DNA, fingerprint and shoes sample databases in the IFS provide clues and evidences for law enforcement agencies. In particular, the DNA database has been effectively used in cases involving human trafficking and missing persons and continuously presents exciting results.

The *Forensic Science and Technology* and *The Chinese Journal of Forensic Medicine* are two journals managed by the IFS, and are well-established platforms for forensic scientists in China to share their experiences with each other.

Research and Training

The IFS has been performing a variety of studies, such as researching on new methods for personal identification, physical evidence tracing and examination, special reagents and devices for sampling and crime scene investigation, and integrated techniques of finding and exploring physical evidence. As a result of these crucial technique breakthroughs and with guidance from experienced researchers, significant achievements have been accomplished, providing more effective methods and tools for solving cases. In particular, the extensive utilisation of DNATyper™15 in China has powerfully and technically sustained the building up of the DNA database and handling of DNA cases.

From 2010 to 2011, the IFS conducted 43 training courses. In total, 3,425 persons from local public security organisations benefited from these courses. Furthermore, from 2004 to the present, more than 400 persons from law enforcement agencies of several countries, including Vietnam, Laos, Cambodia, Myanmar, Pakistan, Bengal, Uzbekistan, Tajikistan and Kyrgyzstan, attended training courses organised by IFS.

Quality Assurance Activities

The IFS actively participates in international proficiency tests, such as the Collaborative Test Service (CTS) and the International Collaborative Exercise (ICE) covering forensic medicine, DNA, toxicology, drug, fingerprint, firearm, questioned document, tool mark and gun mark, with consistently perfect results. The IFS was accredited by the China National Accreditation Service (CNAS) in 2006, and is continuously working towards the goal of providing more reliable output.

CNAS Accreditation.

(continued from page 5)

Cooley, publicly stated that Mr. Blake was acquitted because of the CSI Effect involving gunshot residue.

Considering all the daily crime shows I believe the CSI Effect is real and does have a profound impact on the jury. Any doubt in a criminal trial is grounds for acquittal. I have personal knowledge from credible sources that at the completion of a trial some jurors have been polled to explain their verdict. Many of them have concluded that because of the absence of DNA or other evidence which would implicate the defendant, they believed that the defendant couldn't have committed the crime for which he or she were charged.

The challenges that a crime scene investigator faces today are much more complex than years gone by. In order to meet this challenge today's crime scene investigator must be thoroughly educated, trained, and tested regularly to adhere to strict accreditation standards. A case starts at the crime scene long before the start of the trial. A crime scene investigator has one and only one attempt for perfection at the initial crime scene. In order to "seek the truth" it is imperative that the crime scene investigator answers that challenge. That's reality, not television.

About the Author

Joseph Blozis is a retired NYPD Detective Sergeant who served the City of New York for 29 years, including 22 years within the NYPD's Forensic Investigations Division's Crime Scene Unit and Police Crime Laboratory. He presently is a contracted consultant providing services for federal agencies and global DNA companies. He is an instructor, lecturer, and subject matter expert who has developed training curriculum for the United States Department of State's Antiterrorism Assistance Program for Terror Related Crime Scene Investigations and is the lead instructor for the International Association of Chiefs of Police Comprehensive Crime Scene Course.

Institute of Forensic Science, Ministry of Public Security, Vietnam

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

The IFS Headquarter in Hanoi

Introduction

The Vietnam Institute of Forensic Science (IFS) was founded in 1978 under the Ministry of Public Security as part of the judiciary system. The main functions and missions of the IFS at that time was to conduct crime scene investigations and forensic examinations of samples collected from crime scenes. The IFS was envisioned to attain the highest level of national public security in forensic sciences. Through more than three decades, the IFS has proven to serve an important role in the essential steps of criminal procedure, contributing extensively to the process of solving both criminal and civil cases by producing timely and precise forensic examination results. Generations of IFS officers have also been trained and educated in prestigious domestic and overseas universities in developed countries.

Functions and Missions:

The main missions of the IFS are as follows:

- Perform examinations according to the requirements of criminal proceedings offices, investigators, organisations and individuals.
- Participate in crime scene investigations.
- Research and apply scientific and technical advancements to forensic technique.
- Engage in international collaboration.
- Conduct training in forensic technique for the local police officers and students in police universities.

Organisation Structure

The IFS comprises 8 dedicated divisions and centers, covering a wide range of forensic science areas such as dactylography, ballistics examination, chemical traces examination, acoustics, document examination, forensic medicine, biological examination and drug examination. Also, the national DNA database of criminals was established and continually developed here.

Eight divisions and centers of the IFS:

- Staff and Personnel Division
- Forensic Technique Examination Division
- Traditional Forensic Technique Examination Division
- Document Examination Division
- Forensic Chemistry Examination Division
- Forensic Medicine Examination Center
- Forensic Biology and National DNA Database of Criminals Center
- Drugs Examination Center

Together with the IFS Headquarter in Hanoi, there are 2 sub-institutes based in Danang City and Ho Chi Minh City which assist with less complex cases in the Central and Southern provinces of Vietnam.

The IFS conducts more than 5,000 examinations on about 15,000 samples from law enforcement organisations, and participates in more than 500 different cases of crime scene investigation annually.

International Collaborations

The IFS recognises that cooperation and collaboration programmes are necessary in building up the capabilities of the organisation. The IFS always looks to integration as opportunities to improve ourselves, thus many programmes have been initiated.

Some examples of international collaborations are:

- United Nations Office on Drugs and Crime (UNODC): Training on drug examination skills
- Japan International Cooperation Agency (JICA): Agreement to exchange information and equipments for drug examination
- China Police Department: Expert training and exchange programmes
- International Law Enforcement Academy (ILEA): Expert training and exchange programmes in forensic sciences
- Australia Federal Police, South Australia Police and Victoria State Police: IFS officers frequently attend courses on forensic DNA analysis skills and identification of victims of disasters and terrorism cases
- Laos and Cambodian police: IFS annually deploys training and education programmes, and technique transfers in forensic techniques and forensic sciences.

In particular, we are very proud to be a founding member of the Asian Forensic Sciences Network (AFSN). As an official member of AFSN, the IFS Vietnam looks forward to frequent knowledge exchanges and other collaborations in all aspects of forensic sciences with other AFSN members.

Robot system (TECAN) for DNA Liquid handling in the national DNA database of criminals.

DocuCenter 4500 system for document examination.

Emergence of New Legal Highs: Methiopropamine and Methoxetamine

Ms Tan Ying Ying, Ms Teo Tang Lin, Ms Melody Cai Peiling, Ms Tracy Toh Pei Shi,
Ms Stephanie Lim Hui Jia, Dr Angeline Yap Tiong Whei
Health Sciences Authority, Singapore

Introduction

There has been a global increase in the abuse of new synthetic drugs in the recent few years. These synthetic drugs are often termed as legal highs as they are analogues of existing banned drugs of abuse, having similar chemical structures and psychoactive properties as their illegal counterparts. Due to the widespread and easy availability of the legal highs on the internet, they have become increasingly popular as legal alternatives to the illicit psychoactive substances for the drugs abusers.

The constant emergence of new legal highs has posed challenges to forensic laboratories around the world as many reference materials of such legal highs are often not available. Literature on the analysis of such drugs is also limited. These have made the identification of new legal highs extremely difficult.

In this article, the characterisation study of two new legal highs, methiopropamine and methoxetamine, will be discussed. These two legal highs were each first encountered in Singapore in 2011 as a white powdery substance. Subsequently, these two compounds were detected again in transparent capsules in two exhibits received in 2012.

Methiopropamine or N-methyl-1-(thiophen-2-yl)propan-2-mine, commonly referred to as "MPA", is a thiophene-based analogue of methamphetamine. It possesses similar chemical structure and has a heterocyclic moiety instead of a phenyl group compared to methamphetamine. Methoxetamine or 2-(3-methoxyphenyl)-2-(ethylamino)cyclohexanone, commonly referred to as "MXE" or "3-MeO-2-Oxo-PCE", is an analogue of ketamine. In methoxetamine, the ortho chlorine and the N-methyl group in ketamine have been replaced with a meta methoxy and an N-ethyl group, respectively. Being structurally similar, these two legal highs were believed to be able to exhibit similar psychoactive properties as their illicit counterparts.

In the characterisation study of these two legal highs, several techniques were employed. Accurate mass analysis was performed using time-of-flight (TOF) mass spectrometry while fourier transform infrared spectroscopy (FTIR), gas chromatography/mass spectrometry (GC/MS) and magnetic resonance spectroscopy (NMR) were used in structural elucidation.

Experimental

Reference Material

Commercial standard of methiopropamine and methoxetamine purchased from LGC Limited were used without further purification.

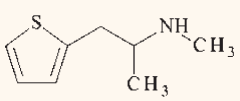

Figure 1: Structure of methiopropamine.

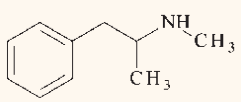

Figure 2: Structure of methamphetamine.

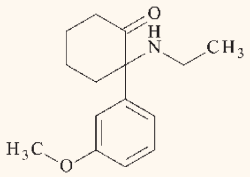

Figure 3: Structure of methoxetamine.

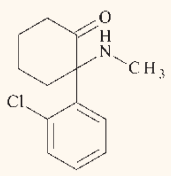

Figure 4: Structure of ketamine.

Instruments

Infrared spectra were obtained using a Perkin Elmer Spectrum GX fourier transform infrared (FTIR) spectrometer in the range of 650-4000 cm^{-1}. Mass spectra were obtained on an Agilent GC/MS system comprising of a 7890A gas chromatograph equipped with a 5975C inert MSD with triple axis detector. Accurate mass analysis was performed on an Agilent 6210 liquid chromatography time-of-flight mass spectrometer (LC-MS TOF) using electrospray ionisation. Both ^{1}H and ^{13}C NMR were recorded on a Bruker 500 MHz NMR spectrometer and the solvent used was deuterated chloroform.

Results and Discussion

The data obtained using the different instrumental techniques were presented.

Methiopropamine

FTIR Analysis:

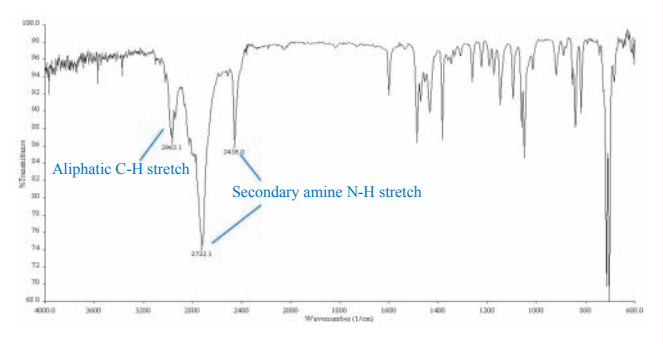

Figure 5: FTIR spectrum of methiopropamine.

GC/MS Analysis:

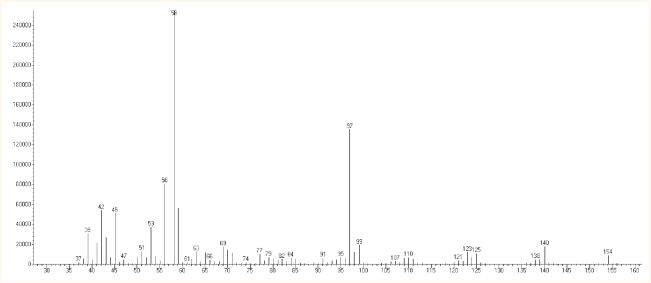

Figure 6: Mass spectrum of methiopropamine.

The major ions observed for methiopropamine using the GC/MS analysis include m/z 140, 125, 97, 58, 56, and the proposed fragmentation pattern of this compound is shown in Figure 7.

LC-MS TOF Analysis:

The measured mass of this compound using LC-MS TOF was 155.0778. This measured value was matched to the mono-isotopic mass of methiopropamine with a mass error of -5.61 ppm, which is within our acceptance criteria of ±10%. The measured and theoretical isotopic abundances of this compound were also matched to ±20%.

NMR Analysis:

The broad singlet in the low field is attributed to the proton (H_i) on the electronegative N atom. The three protons H_g, H_f and H_h, which are attached to the carbons on thiophene, experience the anisotropic effect of the aromatic ring and hence are shifted

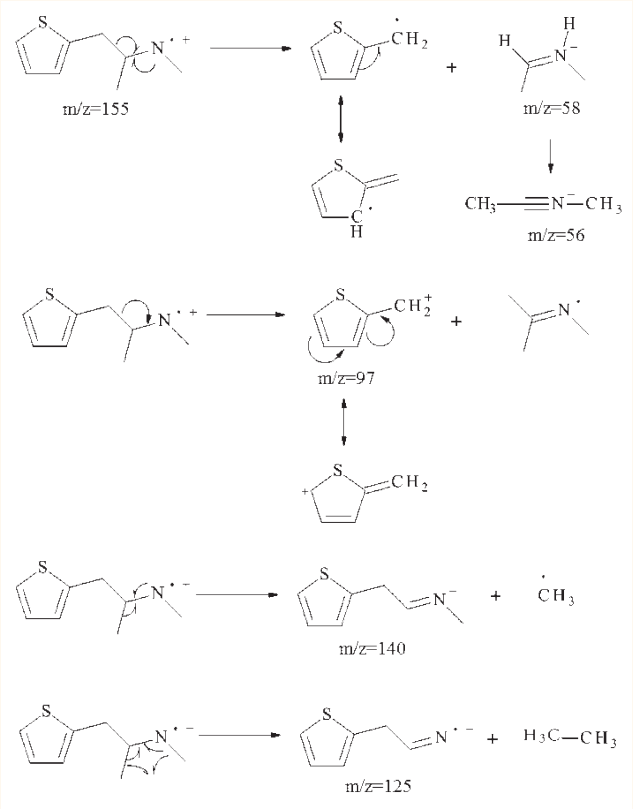

Figure 7: Proposed fragmentation pathways for methiopropamine.

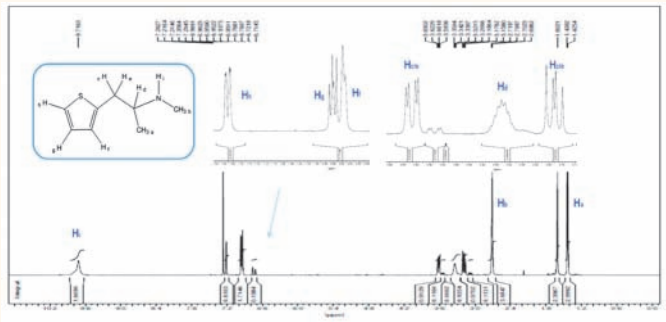

Figure 8: NMR spectrum of methiopropamine

Proton	Chemical Shift	Multiplicity	Integral	Proton Type
H_a	1.43	doublet	3	C-CH_3
H_b	2.68-2.74	multiplet	3	N-CH_3
$H_{c/e}$	3.18	doublet of doublets	1	CH
H_d	3.29-3.40	multiplet	1	CH_3-CH
$H_{e/c}$	3.61	doublet of doublets	1	CH
H_g	6.96	doublet of doublets	1	aromatic CH
H_f	6.93	doublet	1	aromatic CH
H_h	7.21	doublet of doublets	1	aromatic CH
H_i	9.72	broad singlet	1	NH

Table 1: Chemical shifts of protons in methiopropamine.

downfield. Among the three aromatic protons, H_h is at the lowest field because it is attached to a carbon which is adjacent to an electronegative S atom. The coupling constant (^3J) between H_h and H_g is 5.0 Hz, while coupling constant (^4J) between H_h and H_f is 0.9 Hz. H_g is coupled to both protons H_h and H_f with similar coupling constant and shows a clear doublet of doublets. The three protons of N-C-CH_3 (H_a) at the highest field show a doublet due to coupling with the proton Hd (^3J = 6.4 Hz). The three protons of N-CH_3 (H_b) are coupled to the protons H_i and H_d, thus showing a multiplet. The protons H_c, H_d and H_e show a first order AMX pattern. The proton H_c gave a doublet of doublets by coupling to H_d and its diastereotopic H_e; H_e gave a double of doublets by coupling with H_d and its diastereotopic H_c; and H_d gave a multiplet by coupling to H_a, H_c, H_e and H_i.

Methoxetamine

FTIR Analysis:

Absorbance observed at 2684, 2476 and 1726 cm^{-1} indicated the presence of secondary amine and the carbonyl group, respectively.

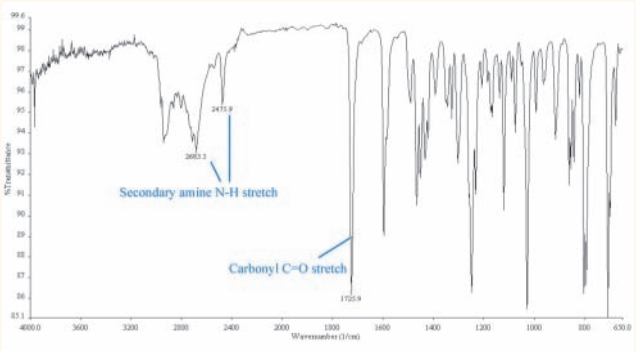

Figure 9: FTIR spectrum of methoxetamine

GC/MS Analysis:

The major ions observed for methoxetamine using the GC/MS analysis include m/z 247, 219, 218, 191, 190, 176 and 134. The proposed fragmentation pathway for this compound has been reported in an article in the *Microgram Journal* [1].

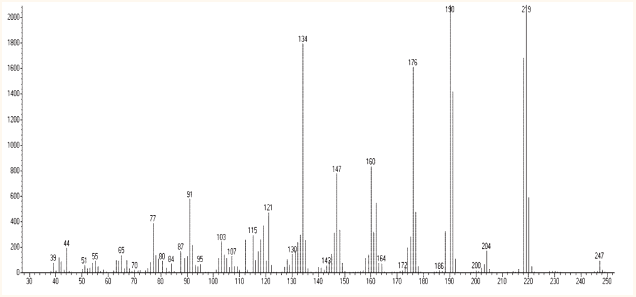

Figure 10: GC/MS spectrum of methoxetamine

LC-MS TOF Analysis:

The measured mass of this compound using LC-MS TOF was 247.1581. This measured value was matched to the mono-isotopic mass of methoxetamine with a mass error of -3.60 ppm, which is within our acceptance criteria of ±10%. The measured and theoretical isotopic abundances of this compound were also matched to ±20%.

NMR Analysis:

The two broad singlets in the low field are attributed to the H_i and H_j protons attached to the electronegative N atom. The four protons H_a, H_b, H_c and H_d which are attached to the carbons on benzene experience the anisotropic effect of the aromatic ring and hence are shifted downfield. The H_a proton couples with H_c and H_d protons with almost same coupling constant. The H_b proton gives a singlet. The H_c and H_d protons are overlapping doublets.

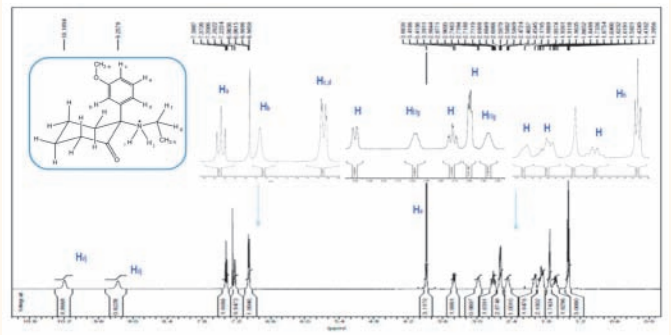

Figure 11: NMR spectrum of methoxetamine

Proton	Chemical Shift	Multiplicity	Integral	Proton Type
H_h	1.41	triplet	3	CH_2-CH_3
H	1.59-1.69	multiplet	1	cyclohexyl
H	1.83-1.95	multiplet	2	cyclohexyl
H	1.96-2.04	multiplet	1	cyclohexyl
$H_{f/g}$	2.41-2.52	multiplet	1	NH-CH_2
H	2.58-2.61	overlapping multiplet	2	cyclohexyl
H	2.72	triplet of doublets	1	cyclohexyl
$H_{f/g}$	2.92-3.05	multiplet	1	NH-CH_2
H_e	3.88	singlet	3	O-CH_3
H	3.41	doublet of doublets	1	cyclohexyl
$H_{c,d}$	6.97, 6.98	overlapping doublets	2	aromatic C-H
H_b	7.22	singlet	1	aromatic C-H
H_a	7.37	triplet	1	aromatic C-H
$H_{i/j}$	9.26	broad singlet	1	NH
$H_{i/j}$	10.19	broad singlet	1	NH

Table 2: Chemical shifts of protons in methoxetamine.

The three N-C-CH_3 (H_h) protons are coupled to H_f and H_g, showing a triplet at the highest field. The three Ar-O-CH_3 (H_e) protons give a singlet in the low field as they are attached to an electronegative O atom. The H_f and H_g protons give two multiplets and are coupled to H_h according to COSY experiment. The remaining peaks are assigned to the protons on the cyclohexyl ring.

Conclusion

The time-of-flight mass spectrometry is a very sensitive technique which allows high mass accuracy determination up to the ppm range. This analytical technique is particularly useful in determining the molecular weight of the compound especially if the parent ion is unstable under the electron impact mode in GC/MS analysis.

The accurate molecular weight together with the mass spectrum obtained from GC/MS analysis allow the structure of each legal high to be elucidated. As the mass spectra of structural isomers tend to be similar, 1-dimensional and 2-dimensional proton NMR is used to further support the molecular structure of each legal high proposed. It was demonstrated from the results obtained from both analyses that the structures of the two legal highs proposed are indeed in agreement. The functional groups present in each of these compounds are further supported by the FTIR results.

Hence, in conclusion, to accurately identify and characterise an unknown compound, a few analytical techniques are required to complement and support each other.

Case Study

Early in 2012, two seizures of transparent capsules were submitted to the laboratory for analysis. The capsules in the first seizure contained a white/purple powdery substance. Approximately 10 mg of the powdery substance in these capsules was first dissolved in 1 ml of purified water, basified with dilute sodium hydroxide solution and then extracted with 2 ml of ethyl acetate. GC/MS analysis of the sample extract revealed the presence of methiopropamine (Figure 12).

The capsules in the second seizure contain a brown powdery substance. For this brown powdery substance in capsules, a total of three different GC/MS profiles were observed in the sample extract prepared using similar extraction technique as mentioned above. Most of the capsules were found to contain methiopropamine, methoxetamine and dimethylphenanthrene (Figure 13), while some capsules were found to contain methiopropamine and dimethylphenanthrene (Figure 14) or in some cases, dimethylphenanthrene alone (Figure 15).

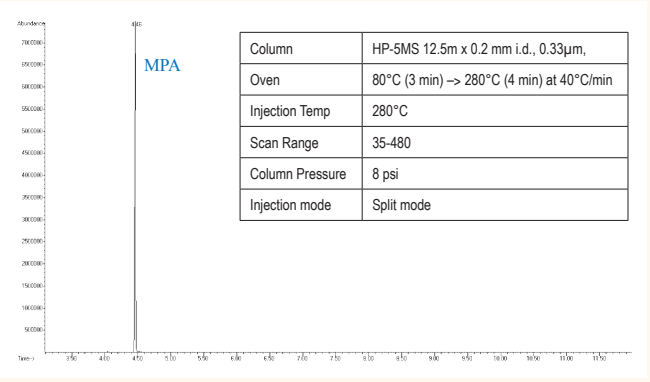

Figure 12: Total ion chromatogram of methiopropamine found in white/purple powdery substance.

In Singapore, many of the Ecstasy tablets containing methamphetamine are also found to contain ketamine. Hence, it is interesting to note that both analogues of methamphetamine and ketamine were also found together in most of the capsules in this seizure. The presence of three different GC/MS profiles observed may be the result of improper mixing of the powdery substance before they are packed into the capsules.

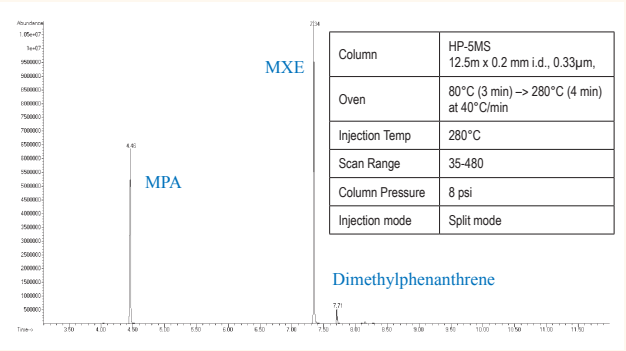

Figure 13: First GC/MS Profile – Methiopropamine, methoxetamine and dimethylphenanthrene detected.

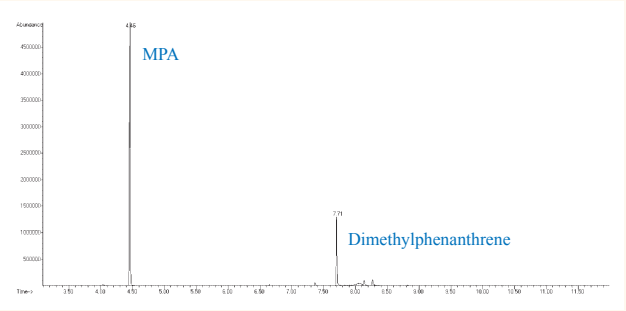

Figure 14: Second GC/MS Profile – Methiopropamine and dimethylphenanthrene detected.

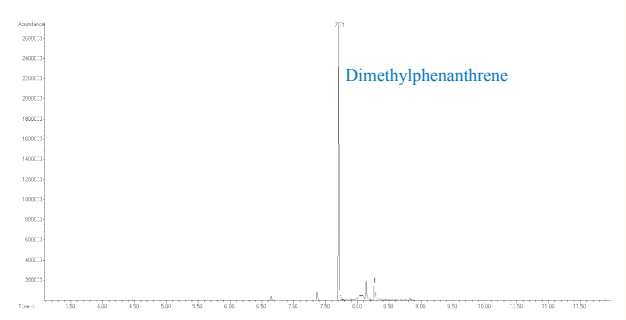

Figure 15: Third GC/MS Profile – Dimethylphenanthrene detected.

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Acknowledgement

The authors would like to thank Dr Lin Qi for performing the NMR analysis and Mr Alex Low for performing the LC-MS TOF analysis as well as interpreting the results.

A Microscopic Study of Microballoons in Emulsion Explosives for Forensic Identifications

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Abstract

This study described and analysed a physical composition of emulsion explosives for identification purposes. Such emulsion explosives, which included Ammonium Nitrate, EDGN, and aluminum, were typically composed of emulsifiers, inorganic oxidisers, hydrocarbon oils, and microballoon sensitisers. Although the mechanism of a detonation based on such microballoons has been extensively studied, there has recently been a growing interest in the embedded microballoons, particularly for forensic sciences. This study explored six brands of emulsion explosives using a conventional optical microscope, a polarized light microscope (PLM), a scanning electron microscope (SEM), and an energy-dispersive spectrometer (EDS). The results showed that five of the six brands of the investigated explosives were consistent with glass-based materials, where a particle-size distribution was in the range of 15-50 micrometres. Consequently, a presence of the microspheres or microballoons was a significant indicator for the presence of the emulsion explosives, yet an absence did not exclude the substance from being an emulsion-type agent.

Introduction

For the past few years, the Thai population, especially those who inhabit the three Muslim-majority southern provinces, has struggled, and at times suffered greatly, from the increasingly escalated violence. In recent years, the violent-crime statistics indicate that separatist violence, caused by the criminal social network of the South Thailand insurgency which started in the early of 2004 has dramatically intensified. Thousands of innocent lives of Government personnel and civilians have been killed by means of several types of high explosives. The high explosives, generally triggered by remote controls, cell-phone transceivers, or command wires, are mostly assembled as improvised explosive devices. Such improvised explosives, which are found at crime scenes, have main charges which sometimes comprise various brands of emulsion explosives containing Ammonium Nitrate, emulsifiers, and sensitisers [1]. Because the emulsion explosives themselves are commonly used in civil works, especially in mining and construction industries throughout the South of Thailand, forensic investigators risk a misleading identification between suspects and innocents' residences. In addition, the morphological and physical appearances of the emulsion explosives may be confused with other slurry explosives and ANFO (Ammonium Nitrate-Fuel Oil) using naked eyes only.

This study therefore aimed at a thorough investigation of the selected brands of the emulsion explosives. We focused on their component, i.e., sensitisers such as glass or plastic microspheres, using different types of microscopic techniques [2, 3].

Materials and Methods

Explosive samples

Emulsion explosives that are commonly used in the civil works were from different regional backgrounds. In this work, six (6) brands of the emulsion explosives were examined:

1. Megablast, Australia
2. Emulex, Malaysia
3. Buster, India
4. Dyno150, India
5. Superpower, India
6. PowerGel, Thailand

The explosive samples were previously seized at several crime scenes from the South of Thailand.

Sample preparation

A pea size of each explosive sample was separately dissolved in 3-5 ml of Pentane in test tubes. The sampled test tubes were centrifuged at 1500 rpm for five minutes, until the floating residues became apparent. The films of the floating residues were carefully dropped on microscopic slides, to be analysed by a transmitted light microscope, a PLM, a SEM, and an EDS.

Instruments

This work focused on the morphology and microstructure of the emulsion explosives. Three conventional microscopes were used to identify the embedded microspheres or microballoons. First, the transmitted light microscope (Olympus BX60) was used to observe the microspheric particles from 10X to 100X magnification. An Optika digital USB camera was used for real-time visual inspections and screenshots. A PLM (NIKON ECLIPSE 50i POL) was then used to observe the microballoons from 4X to 50X magnification. Lastly, the variable-pressure SEM (Hitachi S-3400N) was used to investigate the microballoons at a high resolution of 1000X to 5000X magnification using a 20.0 kV driving voltage. The instrument also had the EDS attachment unit to examine the elemental composition of the observed materials.

Results and Discussion

Based on 3 types of the microscopes, the results could be divided into 3 categories:

1. Transmitted light microscope

With a conventional transmitted light microscope, the small clusters of microballoons were found in five out of six explosive samples, i.e., Megablast, Emulex, Dyno150, Superpower, and PowerGel. Figure 1 illustrates the microballoons found in the

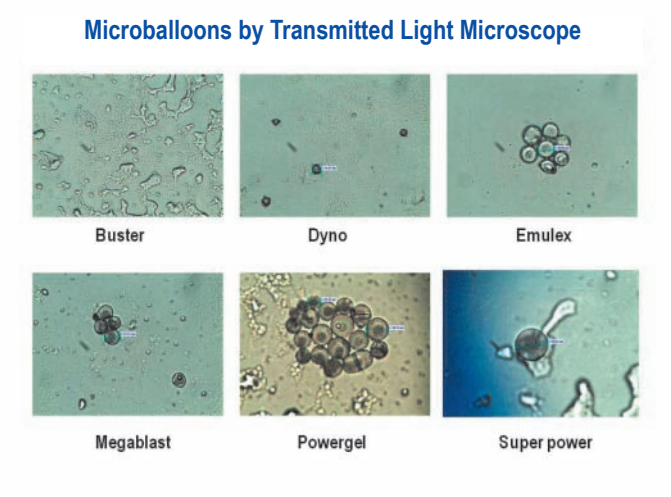

Figure 1: Microballoons as observed by the transmitted light microscope. Buster was the only emulsion explosive without such microballoons.

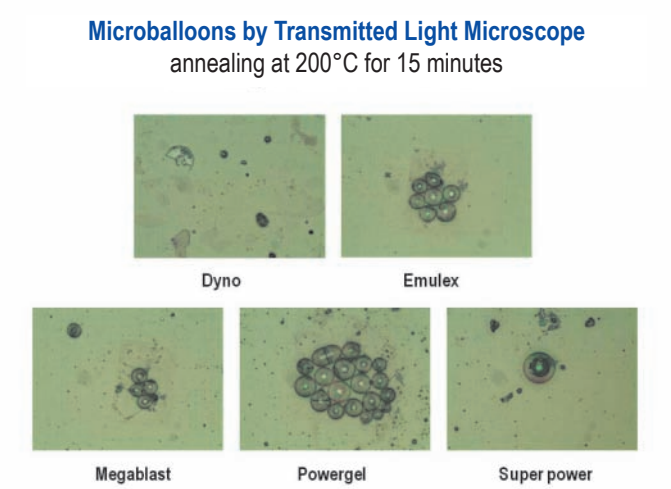

Figure 2: Microballoons, after the annealing treatment, as observed by the transmitted light microscope. The presence of the microballoons indicated that they were made of glass.

five brands of explosives except Buster. These microballoons, although varied in size, demonstrated similar transparent, hollow shells. Buster was the only explosive sample without any traces of microballoons. The authors further investigated the samples with heat treatment in order to determine whether the microballoons were made of glass or plastic. We annealed the explosive samples at 200˚C for 15 minutes. When the authors re-examined the samples with the transmitted light microscope, the microballoons were still observed, as shown in Figure 2, indicating that these microballoons found in the explosive samples were made of glass.

2. *Polarized light microscope*

In order to confirm the results from the transmitted light microscope, the PLM was used to examine the explosive samples. The microballoons were observed in the five emulsion explosives, as shown in Figure 3.

3. *SEM/EDS*

The authors further investigated the microstructures of the microballoons with the SEM/EDS. High-resolution SEM images, as shown in Figure 4, illustrate the apparent microballoons in the isolated manner (Dyno and Superpower), and the small clusters (Emulex, Megablast, and PowerGel.) Figure 4 also shows that the microballoons found in Dyno and Superpower were roughly

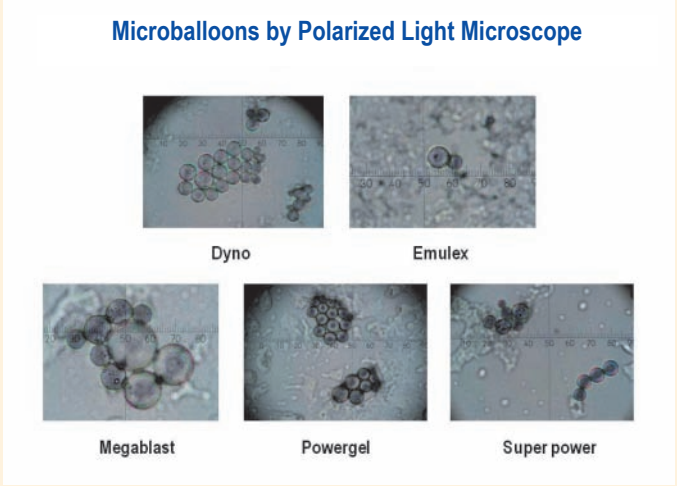

Figure 3: Microballoons as observed by the PLM were found in the small clusters. Buster was the only emulsion explosive without such microballoons.

similar in shape and size, while those found in the other three brands varied in size. From the SEM images, we determined the diameter size of the microballoons at approximately 15-50 µm.

With the EDS attached with the SEM, we were able to determine the chemical elements of the observed microballoons.

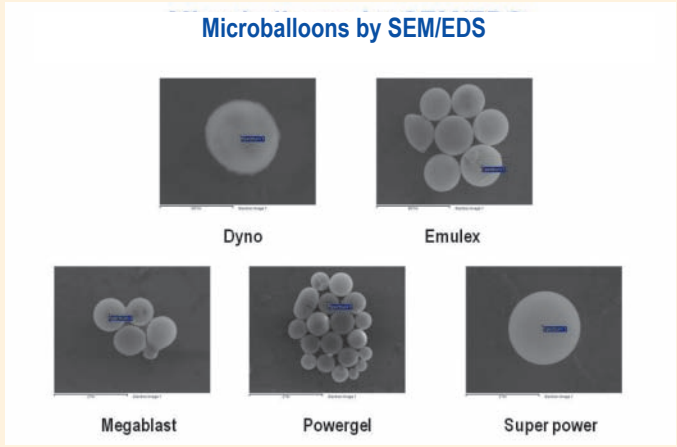

Figure 4: The SEM images of the microballoons. Buster was the only emulsion explosive without such microballoons.

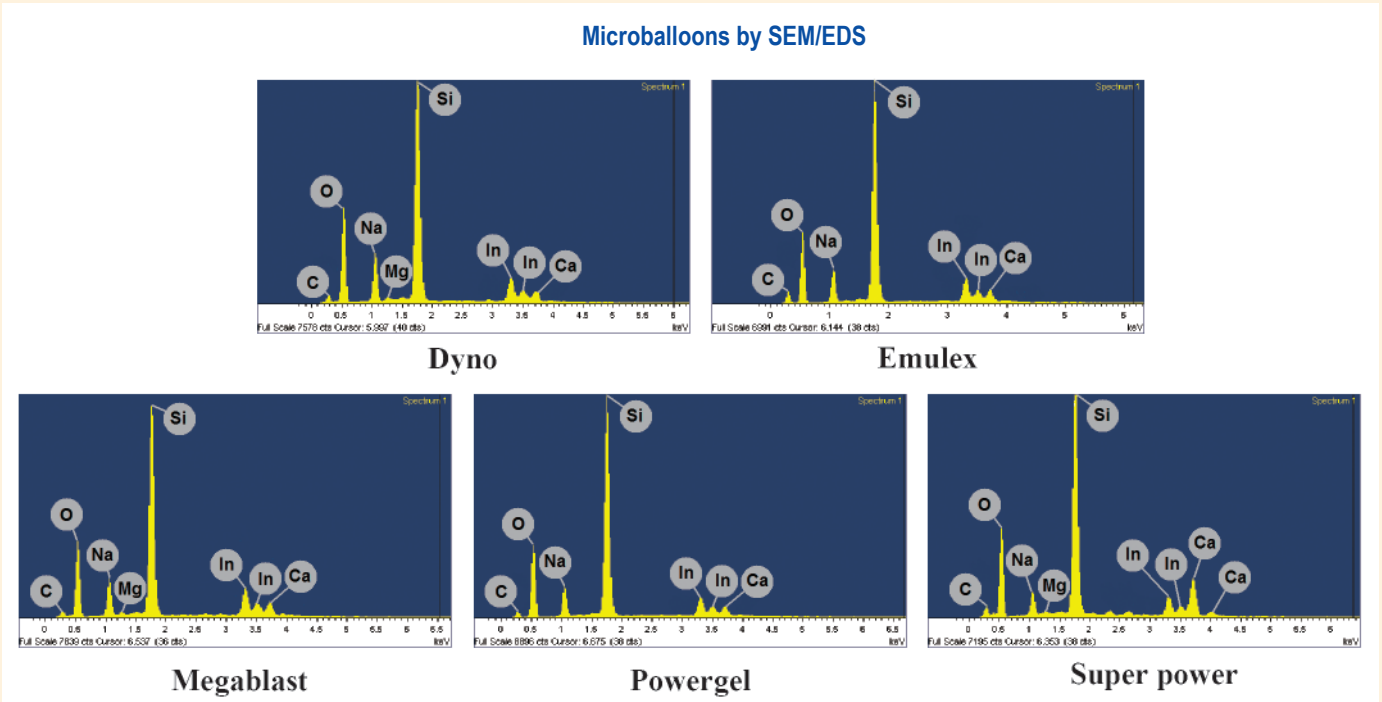

Figure 5: The EDS results of the microballoons as observed in the corresponding SEM images. The results confirmed that the microballoons were made of glass.

Figure 5 and 6 illustrate that the majority of the first three chemical elements were silicon (Si), oxygen (O), and sodium (Na). Such result indicated that the observed microballoons in the emulsion explosives were made of glass.

Conclusion

In an attempt to advance the forensic investigations and analyses of high explosives which have been recently improvised by the South Thailand insurgents, the authors examined several brands of the emulsion explosives, commonly used in the civil works and mining industries throughout the South of Thailand. By primarily focusing at the microballoon sensitisers, the authors employed three different microscopic techniques to observe the embedded microballoons. With the transmitted light microscope, PLM, and SEM/EDS techniques, the results showed that all, but Buster, emulsion explosives contained the microballoons made of glass. The microballoons found in this study were similar in shape and their particle-size distribution varied in the range of 15-50 µm. These results reinforced that the presence of microballoons at pre- or post-blast scenes indicate the presence of emulsion explosives. On the other hand, the absence of microballoons leaves the substance of interest to be further investigated for other types of explosives.

Acknowledgment

The authors gratefully acknowledge the CSI Team at Ingkayutboriharn Army Camp in Nongchik, Pattani Province for their full support and cooperation.

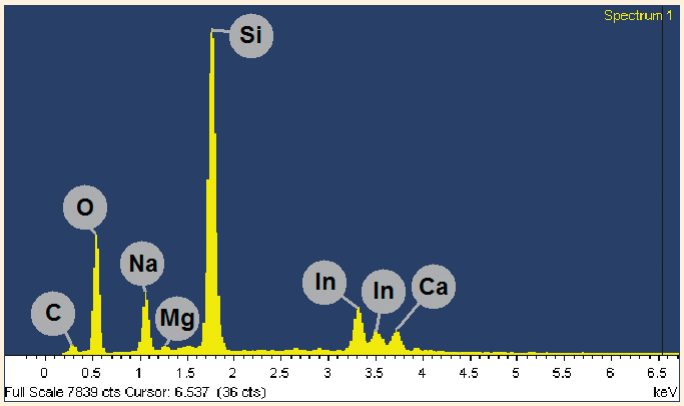

Figure 6: The EDS results of the microballoons found in Megablast explosive sample. The results showed that the majority of the chemical elements were silicon (Si), oxygen (O), and sodium (Na), indicating that they were made of glass. The other chemical elements of indium (In), calcium (Ca), carbon (C), and manganese (Mg) were closely identical to the background spectra originated from the matrix.

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3. TWGFEX Laboratory Explosion Group Standards & Protocols Committee. *Recommended Guidelines for Forensic Identification of Intact Explosives.* TWGFEX. Available at: http://ncfs.org/twgfex/docs/Guide_for_identification_of_intact_explosives.pdf.

Discrimination of Black Cotton Fibre Using a Combination of Conventional Optical Microscopic Techniques and Isotope Ratio Analysis

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Health Sciences Authority, Singapore

Introduction

Fibre has been a well-established form of trace evidence in forensic examination. Cotton is widely used in the manufacture of clothing and black cotton fibres are frequently encountered in fibre cases. Black cotton fibres have limited characteristics and colour is usually the main characteristic for discrimination [1]. Optical microscopy, microspectrophotomety (MSP) and thin layer chromatography (TLC) are common techniques for colour comparison of fibres. Black cotton fibres usually have different coloured hues under the bright field microscope, depending on the class of dyes [2]. MSP has its limitations in differentiating colours if the dyes have similar structures or where fibres are very lightly or heavily dyed, while TLC requires selection of an appropriate solvent system for the extraction of dyes from the fibre.

Over the past decade, isotope ratio analysis has shown to be a powerful technique for applications in forensics [3]. Nic Daéid et al. recently published on the provenance of un-dyed spun cotton fibre using this technique [4]. In this paper, we would like to explore the use of isotope ratio mass spectrometry (IRMS) to complement the conventional techniques for the analysis of black cotton fibres.

Materials and Methods

27 different black cotton garments including shirts, blouse and pants purchased locally were used in this study. The microscopic and fluorescence characteristics of the fibres were examined using a bright field light microscope and a fluorescence microscope (FM) with four filter cubes at wavelengths of 340-380nm, 450-490nm, 355-425nm and 515-560nm.

For carbon isotope analyses, the samples were weighed (triplicate, ~800 µg each) in tin capsules and analysed using

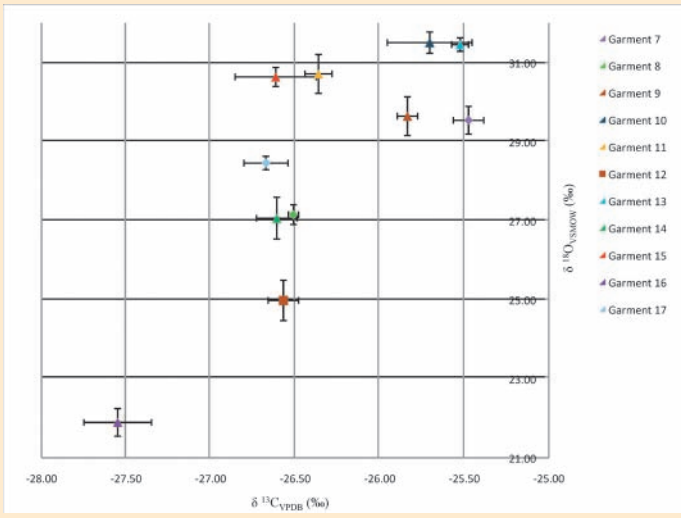

Figure 1: Bivariate plot of $\delta^{13}C$ versus $\delta^{18}O$ values (with ± 2SD ranges) for 11 black cotton fibre samples.

a flash elemental analyser coupled to an isotope ratio mass spectrometer. For hydrogen and oxygen isotope ratio analyses, samples were equilibrated over water for 7 days in a sealed desiccator, then the samples were dried in a vacuum oven over anhydrous phosphorus pentaoxide (Sicapent) for 3 days. After that, samples were weighed (triplicate, ~200 µg each) in silver capsules and dried under vacuum in a desiccator over anhydrous phosphorus pentaoxide (Sicapent) before being analysed using a high temperature conversion analyser coupled to an isotope ratio mass spectrometer. International and in-house standards were used for calibration and quality control purposes.

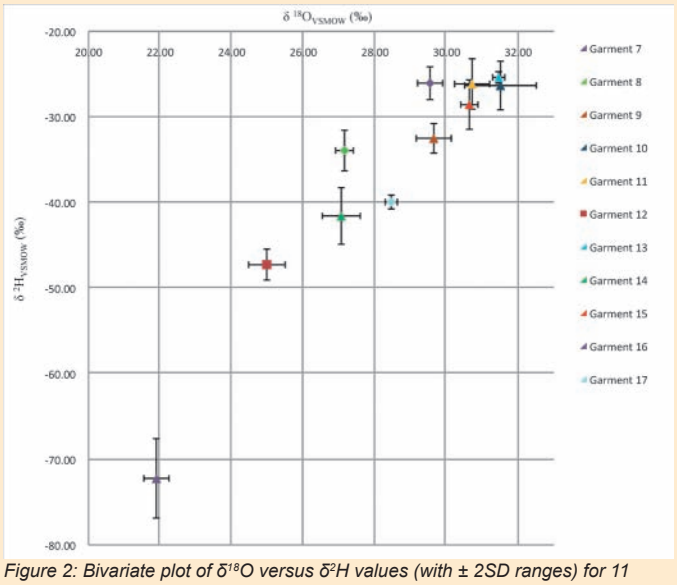

Figure 2: Bivariate plot of δ¹⁸O versus δ²H values (with ± 2SD ranges) for 11 black cotton fibre samples.

Results and Discussion

Using bright field microscopy, the cotton fibres of 27 black garments were classified into 7 groups comprising fibres that appeared dark blue (11 garments), greyish blue (6 garments), black (3 garments), black with reddish tinge (2 garments), grey (2 garments), purplish blue (2 garments) and black with greenish tinge (1 garment). Subsequent examination of the fluorescence of the fibres further classified them into 15 sub-groups marked 1 to 15. To investigate the discriminating power of IRMS, the largest group of 11 garment samples after colour discrimination (dark blue) using bright field microscopy was analysed using IRMS. The results of optical microscopy and IRMS are presented in Table 1.

As the nitrogen content in the black cotton fibres were relatively low, only the carbon, oxygen and hydrogen isotope ratios were used for discrimination of the samples. The total number of possible pairings for these 11 samples was 55. A pair of fibre samples was considered to be isotopically distinguishable when there was no overlap within two standard deviation range of the samples [5]. From the results of the isotope ratio analysis in Table 1 and the bivariate plots (Figures 1 to 3), only 2 pairs (Garment 11 and Garment 15; Garment 10 and Garment 13) out of the 55 possible pairs were indistinguishable. Hence, the discriminatory power of the IRMS technique using a combination of carbon, hydrogen and oxygen isotope ratios was greater than 96%. These two pairs of samples which could not be distinguished by IRMS could be readily distinguished using the FM (refer to Table 1). Thus, the combination of optical microscopy and isotope ratio analysis provided a powerful method for discrimination of black cotton fibres.

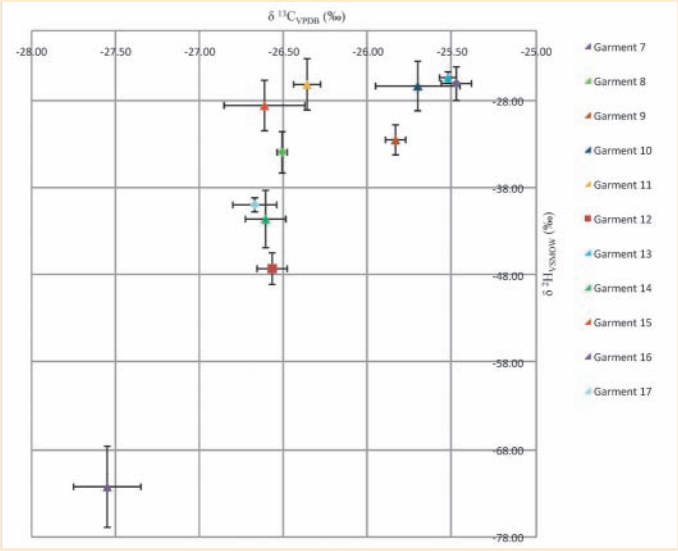

Figure 3: Bivariate plot of δ¹³C versus δ²H values (with ± 2SD ranges) for 11 black cotton fibre samples.

Label	Observation under bright field microscopy	Sub-groups after FM examination	δ¹³C [‰] vs VPDB	δ¹⁸O [‰] vs VSMOW	δ²H [‰] vs VSMOW
Garment 1	Black	1			
Garment 2	Black	2			
Garment 3	Black	3			
Garment 4	Black with greenish tinge	4			
Garment 5	Black with reddish tinge	5			
Garment 6	Black with reddish tinge	6			
Garment 7	Dark blue	7	-25.48 (0.05)	29.54 (0.17)	-26.1 (1.0)
Garment 8	Dark blue	7	-26.51 (0.02)	27.15 (0.12)	-33.9 (1.2)
Garment 9	Dark blue	7	-25.84 (0.03)	29.64 (0.24)	-32.5 (0.9)
Garment 10	Dark blue	7	-25.70 (0.12)	31.51 (0.14)	-26.3 (1.4)
Garment 11	Dark blue	7	-26.36 (0.04)	30.71 (0.24)	-26.2 (1.5)
Garment 12	Dark blue	8	-26.57 (0.05)	24.98 (0.26)	-47.3 (0.9)
Garment 13	Dark blue	8	-25.53 (0.03)	31.46 (0.08)	-25.4 (0.3)
Garment 14	Dark blue	9	-26.61 (0.06)	27.06 (0.26)	-41.6 (1.6)
Garment 15	Dark blue	9	-26.62 (0.12)	30.64 (0.12)	-28.6 (1.4)
Garment 16	Dark blue	9	-27.55 (0.10)	21.89 (0.17)	-72.2 (2.3)
Garment 17	Dark blue	9	-26.67 (0.06)	28.46 (0.08)	-40.0 (0.4)
Garment 18	Grey	10			
Garment 19	Grey	11			
Garment 20	Greyish blue	12			
Garment 21	Greyish blue	12			
Garment 22	Greyish blue	12			
Garment 23	Greyish blue	13			
Garment 24	Greyish blue	13			
Garment 25	Greyish blue	14			
Garment 26	Purplish blue	15			
Garment 27	Purplish blue	15			

Table 1: Results of isotope ratio analysis and optical microscopy.
1.The IRMS results were expressed as δ-values relative to the international standards VPDB (Vienna-Pee Dee Belemnite) for ¹³C and VSMOW (Vienna Standard Mean Ocean Water) for ²H and ¹⁸O.
2.The mean δ value for each garment sample was reported with the standard deviation (1SD) in parentheses.

Conclusion

The above study has demonstrated that the use of conventional optical microscopy coupled with isotope ratio analysis provides a powerful method for the discrimination of black cotton fibres. Moving forward, we are reducing the sample amount required for the IRMS analysis and also studying the effects on the isotope ratios after washing the garment.

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Experiences with the Use of Portable FTIR and Raman in Clandestine Drugs Laboratory Investigations

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Abstract

In clandestine drug laboratories, precursors, chemicals and solvents are used during the synthesis of drugs. At the scene, identification of precursors and chemicals is not sufficient and conclusive by colour tests. Using portable devices such as Attenuated Total Reflectance-Fourier Transform Infrared Spectrometer (ATR-FTIR) and Raman Spectrometer, the forensic chemist will be able to obtain actionable information during the investigation. Colour tests method is limited for identification of certain types of drugs in contrast to these portable devices which not only cover identification of a wide range of drugs but can also provide confirmatory results. Both these techniques also help in identification of precursors and chemicals used in the synthesis of drugs at the clandestine laboratories. Further useful information such as the synthetic route employed can be obtained through the identification of the precursors and chemicals. This article presents the application of FTIR and Raman techniques as a useful tool in the identification of drugs, chemicals and solvents which can provide confirmatory results and indication of possible synthetic route used during the synthesis of drugs at clandestine drugs laboratory.

Introduction

A clandestine laboratory is a laboratory used for the primary purpose of illicitly (illegally) manufacturing controlled substances, such as methylamphetamine, amphetamine, and Erimin 5 tablets. Clandestine laboratories come in all sizes and are found in a variety of locations. The most encountered illicitly manufactured drug across Malaysia is methylamphetamine. Methylamphetamine can be synthesised in a variety of ways, but it is produced most commonly by using either of two primary synthesis methods which differ in the use of the key precursor, 1-phenyl-2-propanone (P-2-P), or ephedrine or pseudoephedrine. Analytical grade and hardware shop chemicals and solvents such as ethanol, acetone, toluene, iodine, red phosphorous, sodium hydroxide, hydrochloric acid, etc. are normally used during methylamphetamine synthesis. Synthetic route employed in the methylamphetamine synthesis can be established through the identification of precursors and chemicals at the site. This piece of information is useful for law enforcement agencies to control the accessibility to those precursors and chemicals in the country.

Rapid and precise identification of drugs and chemicals is one of the key tasks of the forensic chemist, especially with the marked increase of clandestine drug laboratories. Colour test, which is a conventional method and presumptive test with Marquis, Simon and other reagents, assists the forensic chemist in determining some of the drugs, but are not effective in the identification of a broad range of drugs and precursors. Furthermore, a conclusive result cannot be achieved at the scene solely based on the result obtained from colour tests. Similarly, the identification of most precursors and chemicals cannot be obtained by colour test. For this purpose, portable FTIR and Raman spectrometers might be used to help the forensic chemists with investigations at the crime scene.

FTIR and Raman serve as complementary and confirmatory analyses for many samples. Being non-destructive testing techniques, they maintain sample integrity. Both FTIR spectroscopy and Raman spectroscopy measure the interaction of energy with the molecular bonds in a sample of an unknown liquid or solid material [1, 2]. Additionally, portable Raman has the advantages over other portable device as it can be operated through containers such as plastic bags and glass bottles [3]. FTIR and Raman are vibrational techniques where light or laser pass through the object. FTIR measures how much an infrared light is absorbed by the bonds of a vibrating molecule. In comparison, Raman measures the energy that is emitted or scattered when an intense single wavelength laser is focused on a sample [1, 2]. When used together, FTIR and Raman spectroscopy can provide confirmatory results and a broader range of unknown substance identification. FTIR technique is less suitable to identify substances in solution form (such as in water due to the strong infrared signal from water) and white or light coloured powder, whereas Raman technique is less suitable to identify dark-coloured, highly fluorescent materials and most pure metals and elemental substances. Some materials that cannot be identified by Raman are amenable to identification by FTIR, and vice versa [4]. Both these techniques are not suitable for trace identification.

Materials and Methods

Infrared spectra were obtained on a HazMatID instrument (Smiths Detection), with instrumental parameters as summarised below in Table 1. Spectra analysis was undertaken using Qual ID software (Smiths Detection). Searches were performed against custom and commercial libraries including forensic drugs, drug precursors, common chemicals, toxic and industrial chemicals and commercially available common “white powders”.

Resolution:	4 cm^{-1}
Scans:	64
Background Scans:	64
Spectral Range:	4000-650 cm^{-1}
Detector Type:	TGS
Y Axis Units:	Abs
ATR Crystal:	Diamond

Table 1: Instrumental parameters for HazMatID.

Raman spectra were obtained on a FirstDefender RM instrument (Thermo Scientific), with instrumental parameters as summarised below in Table 2. Spectra analysis was undertaken using GRAMS® software. Searches were performed against a substance library which included explosives, toxic industrial chemicals (TICs), toxic industrial materials (TIMs), chemical warfare agents (CWAs), narcotics, precursors, “white powders” and more.

Use Mode:	Point-and-shoot direct or through translucent containers
Spectral Resolution:	7 to 10.5 cm^{-1}(across range)
Spectral range:	250 cm^{-1} to 2875 cm^{-1}
Exposure:	Manual, Automatic modes (5ms minimum)
Laser Output:	Power Adjustable, 75 mW, 125 mW, 250 mW

Table 2: Instrumental parameters for FirstDefender RM.

The identification of illicitly manufactured drugs is certainly an important facet of clandestine laboratory investigations, but the identification of precursors and chemicals which indicate the synthetic route employed during the synthesis of the drug is equally important. A list of samples most encountered at clandestine laboratories was analysed with both instruments. These included drugs as well as precursors and chemicals.

Results and Discussion

Table 3 outlines the respective suitability of FTIR and Raman for the most common materials encountered at clandestine drug laboratories. Figure 1 illustrates FTIR and Raman spectra

Type	Examples	FTIR	Raman
Drugs	Methylamphetamine	Y	Y
	3,4-Methylenedioxymethamphetamine (MDMA)	Y	Y
	Cocaine	Y	Y
	Nimetazepam	Y	Y
	Ketamine	Y	Y
Precursors	Pseudoephedrine	Y	Y
	Ephedrine	Y	Y
	Safrole	Y	Y
	1-Phenyl-2-propanone	Y	Y
	3,4-Methylenedioxyphenyl-2-propanone	Y	Y
Chemicals	Caffeine	Y	Y
	Red phosphorous	N	Y
	Iodine	N	N
	Sodium hydroxide	Y	Y
Solvents	Acetone	Y	Y
	Toluene	Y	Y
	Chloroform	Y	Y
	Methanol	Y	Y
	Sulphuric acid	Y	Y
	Hydrochloric acid	Y	N

Table 3: List of the most common materials encountered at clandestine drug laboratories in Malaysia and suitability of FTIR and Raman for their analysis.

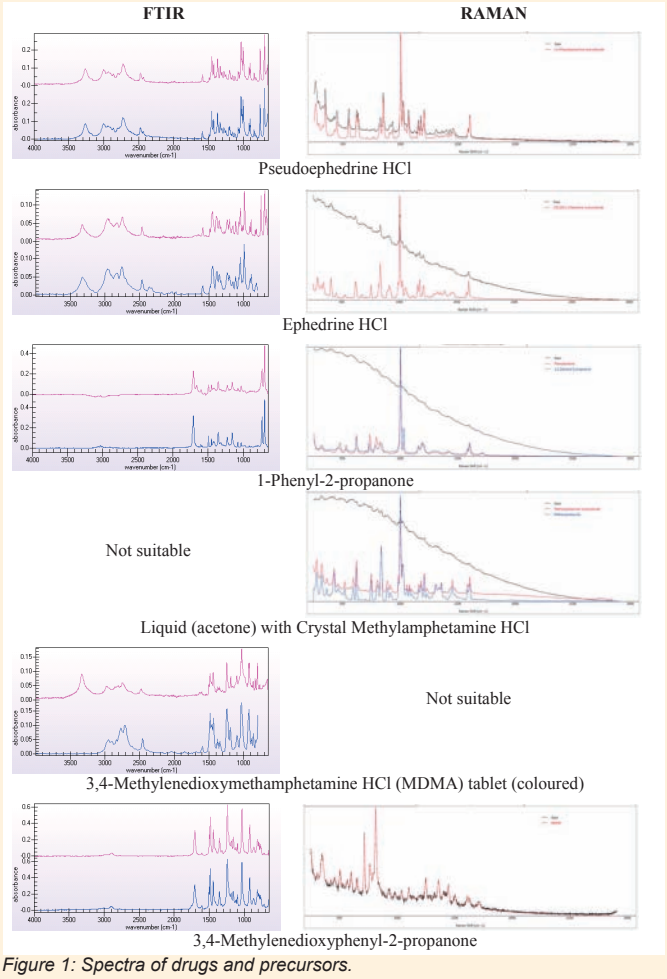

Figure 1: Spectra of drugs and precursors.

of clandestine laboratory samples of several types of drugs and precursors. Likewise, Figure 2 illustrates FTIR and Raman spectra of clandestine laboratory samples of chemicals and solvents.

Both the FTIR and Raman spectra are displayed with two different colours for easy differentiation between library and sample. For the infrared spectrum, there are two lines with the blue line representing the library spectrum and the purple line representing the sample spectrum. There are also 2 or 3 lines for the Raman spectrum with the red and blue line representing the library spectrum and the black line representing the scan data.

FTIR and Raman are routinely used for solid and liquid analysis, as discussed in previous sections of the article. In the context of material identification with FTIR and Raman,

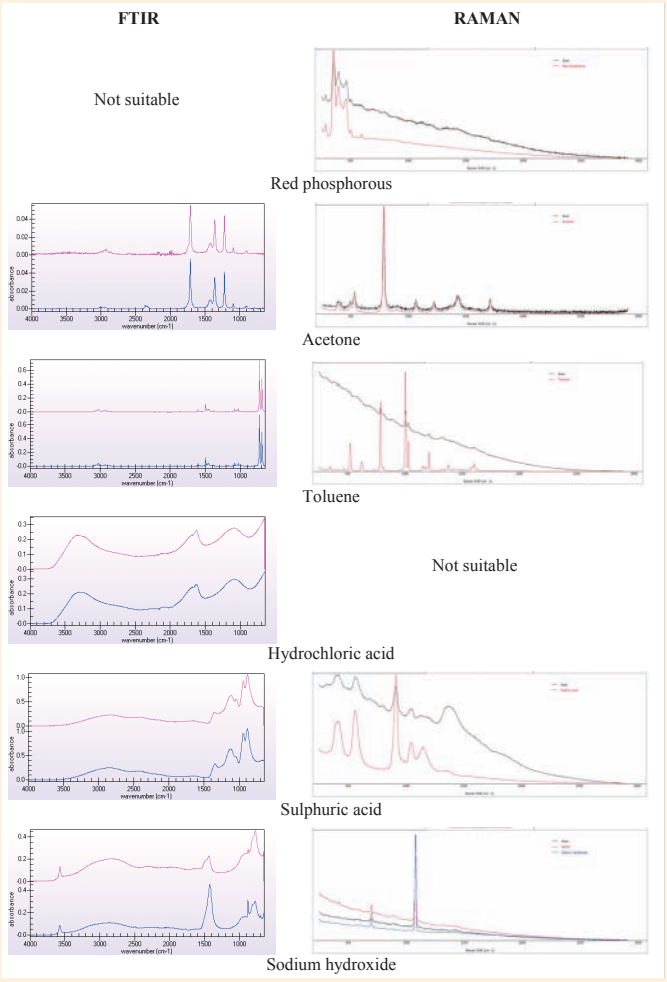

Figure 2: Spectra of chemicals and solvents

there are several practical aspects that govern applicability. While most pure drugs can be measured with either FTIR or Raman, some materials are better suited for measurement by a particular technique. For example, some materials can be very difficult to identify with Raman systems due to overwhelming fluorescence. Figure 1 shows a Raman spectrum of coloured tablets of MDMA which failed to produce a positive match, but fluorescence is obviously not a limitation in FTIR and gives a positive match with MDMA. By contrast, aqueous samples containing drugs are difficult to measure with FTIR due to solvent or water interference. Solvents or water are rarely an interferent in Raman analysis. This is shown in Figure 1 where Raman gives a positive match even with low quality spectrum for crystal methylamphetamine with acetone as solvent medium, whereas FTIR is limited to a positive match only with crystal methylamphetamine and not with a mixture of acetone. However, caution should be taken with energetically sensitive materials such as red phosphorous due to an ignition risk when measured by Raman with high power excitation. To overcome this problem, analysis should be performed on a solution form of a small amount of sample with water in a transparent uncapped vial. Raman technique has the ability to operate in a point and shoot mode through semi-translucent containers. Sometimes the quality of some Raman spectra are low as shown in Figure 1 (ephedrine and P-2-P) and Figure 2 (toluene) due to inference from semi-translucent containers and also the florescence inference from the sample.

Conclusion

FTIR and Raman can be utilised by the forensic chemist as a first responder for the identification of a whole range of drugs, precursors, chemicals and solvents at clandestine drug laboratory investigations. Through the application of portable device, fast and more accurate identification of chemicals and solvents at scene can be obtained. Either of the techniques may be employed by the forensic chemist in the identification of chemicals and solvents at a clandestine drugs laboratory.

Tactically, Raman has the advantage of being non-contact and capable of performing analysis of materials contained in clear and coloured transparent and translucent containers. With caution while handling samples, the Raman technique is also ideal for inorganic materials such as red phosphorous for enabling clear and unique identification of compounds. In cases where data collection may be hindered by fluorescence interference caused by dyes in Raman, FTIR is able to collect data as well, underlying the complementary nature of both techniques. Raman can be the supplementary choice of technique to use with the more common use of FTIR at clandestine drugs laboratory for identification of inorganic, water-based materials and white powders. Information about hazardous materials (such as NFPA diamond) can be accessed and viewed onsite via the portable Raman result screen. This will help the forensic chemist to handle investigations with care.

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A Cost-effective Method for Screening of Y-SNP Marker at M95 locus and its Utility as a Marker in Southern Thai Minorities

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Abstract

This paper demonstrates the single nucleotide polymorphisms (SNPs) by gel electrophoresis as a cost-effective method for screening the Y-SNP marker at M95 locus and its distribution frequencies in southern Thai minorities. The result from gel electrophoresis method is well congruent with the result from direct DNA sequencing, and is thus valid for Y-SNP screening and useful for a large number of samples. Distribution frequencies of Y-SNP M95 in each Thai minority has been compared and reported.

Introduction

Analysis of single nucleotide polymorphisms on the Y chromosome (Y-SNPs) is a good option for degraded, destroyed, mixed and minute DNA samples [1, 2], as it can achieve additional information which STRs fail to obtain, and is easy for analysis. Y-SNPs are the most useful genetic markers for reconstructing male lineages due to lack of recombination and low mutation rates. The challenge was to develop a set of polymorphic Y-SNP markers that are specific to the Thai population to be used for haplogrouping from low quality DNA samples. The objectives of this work were to demonstrate a cost-effective method for screening the Y-SNP marker at M95 locus and to report its distribution frequencies in southern Thai minorities. The Y-SNP marker at M95 locus was selected for this purpose, as it is commonly found in Asians and Southeast Asians including haplogroups in Thailand, India, Cambodia, Malaysia, China, and Indonesia [3, 4].

Materials and Methods

Population samples

Buccal swabs of 384 unrelated males in various occupations e.g. university student, soldier, government officer, and merchant, living in the southern provinces of Thailand were collected. Each male was asked to give ethnic details of his parents and grand-parents, and written informed consent was obtained before sampling. According to their ethnic origins, the samples were divided into 5 groups, including Thai (130), Malay Muslim (97), Chinese (110), Thai-Malay Muslim (7), and Thai-Chinese (40). This study was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University.

DNA extraction

DNA of each sample was extracted using two different methods for confirmation; including 5% chelex 100 and a commercial genomic DNA extraction kit. The DNA concentration was quantified using real-time PCR.

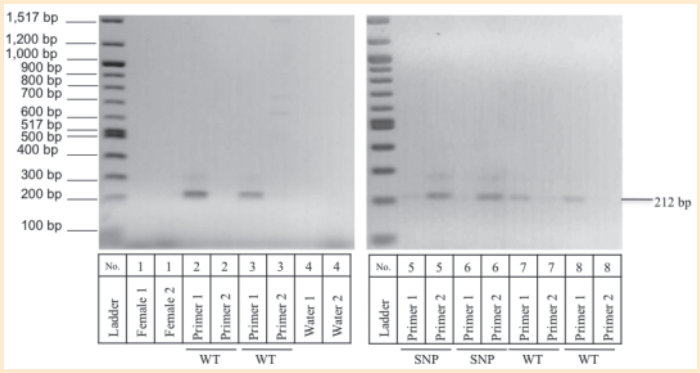

Figure 1: Agarose gel image showing PCR products of M95 locus. Each experiment was repeated for at least 3 times. Wild type primer (1), SNP primer (2), wild type (WT).

Y chromosome PCR amplification

Ten ng of DNA was amplified at the M95 locus on the Y chromosome using a pair of either a wild type forward primer or a SNP forward primer, and a reverse primer. The sequence of the wild type forward primer was (5'-3') GAT AAG GAA AGA CTA CCA TAT TAG TGC, the SNP forward primer was (5'-3') GAT AAG GAA AGA CTA CCA TAT TAG TGT and the reverse primer was (5'-3') GGG TGG GTG TGT TTG AAG G. Each reaction mixture containing 0.2 mM each dNTP, 1.5 mM MgCl2 2.5 U/µl Taq DNA Polymerase in 1X PCR buffer (Invitrogen) and 0.5 µM primer pair was prepared. The PCR was performed using 36 cycles of 94°C for 45 s, 60°C for 30 s and 62°C for 30 s, 72°C for 1 min 30 sec and final extension at 72°C for 10 min.

Y-SNP analysis

The PCR products were separated by agarose or polyacrylamide gel electrophoresis. The results were examined and labeled as wild type or SNP depending on the appearance of the DNA bands. Subsequently, 45 wild type and SNP PCR products were randomly selected for genotyping by direct DNA sequencing. The genotypes of newly sequenced DNA were determined by blasting to the public database (www.ncbi.mlm.nih.gov).

Results

The PCR products, which had M95 wild type allele, showed intense DNA band (212 bp) on the gels when using the wild type forward primer, but showed faint DNA band when using the SNP forward primer. At the same time, the DNA, which had M95 SNP allele, showed faint DNA band when using the wild type forward primer, but intense DNA band when using

the SNP forward primer (Figure 1). The above result from gel electrophoresis method is well congruent with the result from direct DNA sequencing, in which the haplotype of the Y-SNP at M95 locus was T instead of C (Figure 2). Some 113 of 384 PCR products were SNP genotype (29.42%). The distribution of Y-SNP M95 in each Thai minority is shown in Table 1. The statistical comparison between the minorities is shown in Table 2, and the comparison of Y-SNP M95 distribution found in this study and others is shown in Table 3.

	Thai (n=130)	Malay Muslim (n=97)	Chinese (n=110)	Thai-Malay Muslim (n=7)	Thai-Chinese (n=40)
SNP Count	40	34	33	1	5
Frequency of SNP in the whole population (%)	10.42	8.85	8.59	0.26	1.30
Frequency of SNP in each sub-population (%)	30.76	35.05	30.00	14.29	12.50

Table 1: Distribution of Y-SNP M95 in southern Thai minorities (n=384).

Comparison between	p-value	Comparison between	P-value
Thai/Malay Muslim	0.622	Chinese/Thai-Malay Muslim	0.016*
Thai/Thai-Malay Muslim	0.011*	Chinese/Malay Muslim	0.000*
Thai/Chinese	0.898	Thai-Chinese/Thai-Malay Muslim	0.847
Thai/Thai-Chinese	0.007*	Thai-Malay Muslim/Malay	0.003*
Chinese/Thai-Chinese	0.010*	* = Statistical significance	

Table 2: Statistical comparison of Y-SNP M95 frequency between southern Thai minorities

	Frequency (%)		
	This study	Other study	Reference
Thai	30.76	45	Li, D et al., 2010
Malay Muslim	35.05	27.8 34.37 7.7	Kayser et al., 2003 Karafet et al., 2005 Li, D et al., 2010
Chinese	30.00	2.8 11.72 0 6.62	Kayser et al., 2003 Karafet et al., 2005 Weibo et al., 2008 Han, S.Y et al., 2009
Thai-Malay Muslim	14.29	NA	NA
Thai-Chinese	12.50	NA	NA

Table 3: Comparison of SNP frequency between this study and others.

Discussion

In order to develop a set of SNP markers that are specific to the Thai population, a lot more efforts and investments would be needed. A more cost-effective method for screening of Y-SNP markers has been developed. The results of the gel electrophoresis method have been demonstrated to be congruent with genotyping by direct DNA sequencing.

Figure 2: Blast results from 2 different samples; wild type (above) and SNP (below). The red arrows indicate the SNP site. In the SNP sample, the base was changed from Guanine to Adenine (reverse DNA strands).

Frequency of Y-SNP at M95 locus in southern Thai population found in this study is 29.42%, while it was reported as 45% in northern Thai [5]. When the frequencies of Y-SNP M95 in southern Thai minorities were compared, it was interesting to see significant differences between minorities, as shown in Table 2. The observed Y-SNP M95 occurrence in Thai population groups was shown to vary by geographic locations and among the various ethnic groups. The distribution of Y-SNP M95 in southern Thai populations (29.42%) is compared to other Asian country such as India (22.24%), Cambodia (23.1%), Malaysia (34.37%), China (11.7%), and Indonesia (27.8%) [3, 4]. This is consistent with reported studies of Y-SNP M95 occurrence being restricted to the southern populations of East Asia [3-5].

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Value of a Local Vehicle Paint Database

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Introduction

Databases are commonly used in forensic science for purposes of narrowing down the possible source of a sample and to provide information on the frequency of occurrence of a sample. Databases comprising local samples are often most useful and relevant in the local context.

The Vehicle Paint Database (VPD) is the first local vehicle paint database started in Singapore in 2008 for criminal and counter-terrorism investigations. Unknown paint fragments recovered from hit-and-run traffic accidents or vehicle bomb attacks can be searched against the VPD for possible associations.

Vehicle paint generally consists of three to four layers of paint, each with different chemical compositions that serves different functions. Refer to Figure 1 for the types of layers in a typical vehicle paint sample.

Samples in the Database

Paint samples in the VPD are collected progressively from local repair shops and vehicles involved in traffic accidents. Physical characteristics of each sample, including the layer colour and layer sequence are examined. Chemical data of

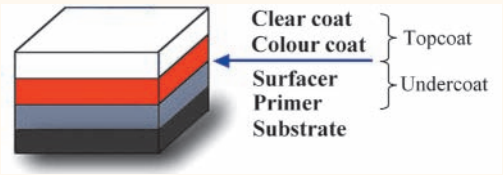

Figure 1: Layer Sequence of a typical vehicle paint.

every layer are obtained using Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy. Currently, the VPD has 1181 samples, of which 750 have been analysed. Most of the samples analysed were of Toyota, Honda and Nissan makes as these makes constitute more than 50% of the cars in Singapore. Refer to Figure 2 for the make distribution of the 750 samples examined.

Findings from VPD

Based on the samples examined and their chemical data obtained, the surfacer layer has been found to be the most distinctive, and can usually be used to identify the make of a car. The colour coat of black and champagne cars and the primer layer of plastic vehicle parts were found to be distinctive to samples recovered from Honda, Toyota and Nissan vehicles. For Nissan and Honda, their models could be classified into two main groups: (a) For Nissan, samples recovered from Sylphy and Latio models belonged to one group and Sunny, Cefiro and Presage models belonged to another; (b) For Honda samples, Civic, Stream and Odyssey models belonged to one group and Jazz, City and CRV models belonged to another. European cars could be distinguished from Asian cars, particularly from the undercoats.

Blind Tests

Blind tests carried out on 45 samples to test the capability of the VPD revealed that about 60% could be narrowed down to the correct make of an unknown vehicle. These 45 samples were new samples yet to be added to the database and they were sampled from small panels or fragments of multi-layered paint collected from local repair shops or vehicles involved in traffic accidents. For the blind tests, the identities of these 45 samples were not made known ("blind") to the examiner. The 45 blind test samples were searched against 500 samples in the VPD using the searching function of FT-IR software, which generates a list of hits for every layer of the unknown samples. The spectra of the list of hits were reviewed and conclusions on the possible make of each sample were based on the combined information obtained from the multiple layers.

Although the remaining 40% of the samples could not be narrowed down to a make, most of them could correctly eliminate the makes present in the database as possibilities, as they were not represented in the database. None of these samples gave the incorrect make.

Using the same unknown samples, a comparison was made with the Royal Canadian Mounted Police's Paint Data Query

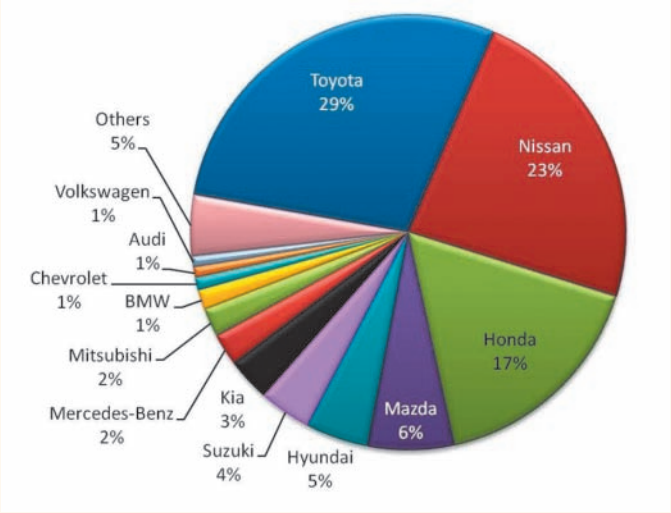

Figure 2: Pie chart showing the distribution of makes of samples examined.

(PDQ) [1, 2], a vehicle paint database used in North America. Compared with the local database, the samples that could be narrowed down to the correct make were found to be less using PDQ. This result is expected as the PDQ database is not representative of the local vehicle population, which makes the PDQ less relevant in the local context. Vehicles supplied to North America mostly come from a different source as those supplied locally, resulting in differences in chemical compositions.

Case Studies

In the early stages of setting up the database when the VPD had limited samples comprising mainly Japanese makes, it was still useful in narrowing down the correct make of a missing car which left behind paint fragments at a murder scene when combined with data from broken plastic fragments and tyre track marks left behind at the scene. The missing vehicle was eventually found by investigators. Refer to Figure 3 for more details of this case.

In recent months, the VPD has been utilised to provide investigative leads for hit-and-run accidents. Vehicle paint smears may be encountered on the clothing of a pedestrian involved in a traffic accident due to the forceful impact between the pedestrian and a vehicle. In one case, a thin paint smear found on the clothing of a hit-and-run victim was searched against the VPD. Together with the broken plastic fragments of the signal lamp recovered from the scene, the possible vehicle make was narrowed down. This provided a new lead to investigators, who were initially misled by inaccurate eye-witness accounts on the colour and type of vehicle.

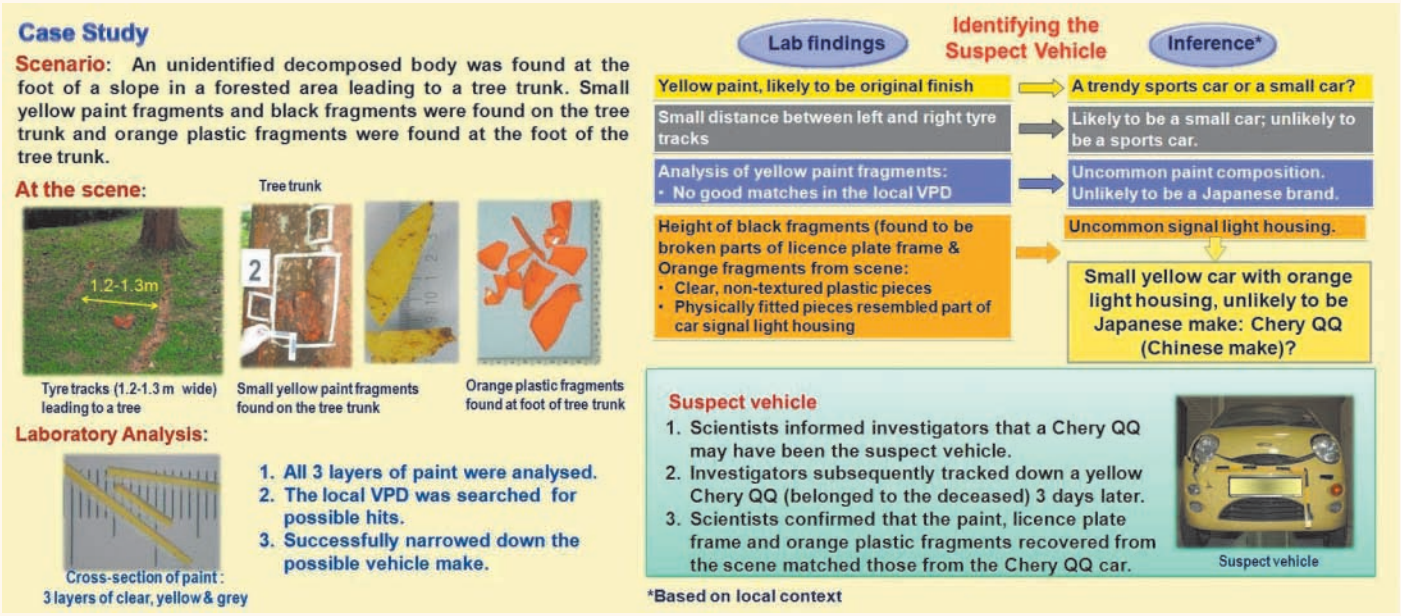

Figure 3: Details of case study illustrating how the VPD helped to narrow down the vehicle make.

(continued on page 16)

Challenges

Building up an exhaustive database can be a challenge as makes or models not represented in the database would place a limitation on the success rate of correctly identifying an unknown sample. Without good representation of the vehicle population in the database, it will be difficult to assign the degree of certainty to the makes that the VPD could narrow down. Input of samples into the VPD has to be ongoing to keep the database relevant, up-to-date and representative of the local vehicle population.

In many of the actual cases encountered, only one or two layers of vehicle paint were transferred and they sometimes occurred as fragments with distinct layers or smears with adjacent layers co-mingled. Hence, with each unknown sample, the degree of certainty of correctly identifying the make of the vehicle paint would be dependent on the number of layers present, the uniqueness of each layer, and the severity of contamination from adjacent layers and/or other materials.

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A Comparison Study on the Significance of Stroke Formations in the Examination of a Chinese Signature

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Abstract

This paper reports a comparison study on the features used to support the conclusions of signature examination among 109 laboratories around China. Each study participant was asked to offer no more than 10 features which can support the conclusion in the examination of a Chinese signature. These features were counted, categorised and analysed. The results demonstrated that stroke formation in Chinese signature examination is very important due to the complex structure of Chinese characters.

Introduction

Chinese characters, compared with English letters, are relatively complicated in their structures. Unlike English words which are composed of linearly arranged discrete units [1], Chinese characters are based on ideograms, which incorporate three important elements: shape, pronunciation and meaning. How the characters' features and outline are presented when written are determined by the writer's mind and aesthetic sense. Regardless of the number of strokes they are composed of, each character is written stroke by stroke and designed to fit into the same imaginary square frame [2]. With globalisation and increased communication among countries in recent years, the Chinese language is not only used in China and other Southeast Asian countries, but also in countries all over the world. Therefore, it is highly important to introduce the methods of Chinese handwriting identification to the world. For this purpose, standard guidelines must be put in place, an area into which we are investing our efforts.

Chinese characters that existed four or five thousand years ago came from the Oracle Bone script (Jia Gu Wen), and have since evolved over the years, going through various scripts such as the Small Seal Script (Xiao Zhuan) and the Official Script before developing into today's fonts. In accordance with the classification of handwriting features which are closely linked to the structures of Chinese characters, eight types of Chinese character features have been recognised, including: (1) overview features (comprising writing skill, the font size, and the font style, etc.); (2) arrangement features (comprising word spacing, line spacing, page margins, etc.); (3) writing characteristics (comprising specification, abbreviations, foreign words, etc.); (4) wrong writing features (comprising writing a wrong character, missing strokes, additional strokes); (5) proportion features (the arrangement of units in one character); (6) stroke sequence; (7) stroke formations; and (8) writing impression characteristics (writing trace) [3].

In Chinese handwriting (signature) identification, the examiner's skill and experience play an important role in the examination process. It is also important to consider the occurrence frequency of the eight types of features mentioned above. For this reason, research such as the quantitative statistics of stroke sequences was conducted [1]. This paper presents an analysis by occurrence frequency of the characteristics of a Chinese signature based on comparisons around China. It is recognised that stroke formation is of high value in Chinese signature examination.

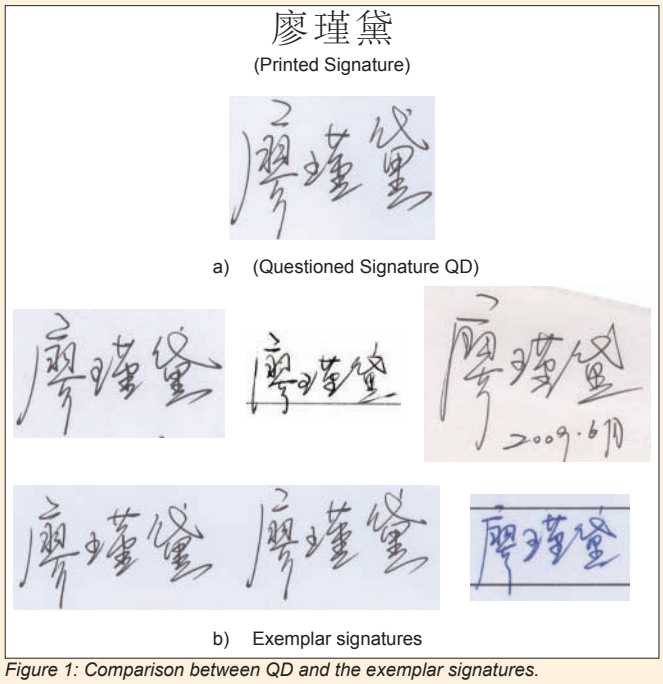

Figure 1: Comparison between QD and the exemplar signatures.

Materials and Methods

A Chinese signature of "廖瑾黛" (Liao Jindai, "廖" is the family name, and "瑾黛" is the first name) was designed as the questioned signature (marked as "QD", shown in Figure 1a). In the comparisons, 109 participants were provided with five pieces of documents containing a total of 6 comparison signatures (Figure 1b) and required to identify whether the QD signature was written by the person who wrote the signature "廖瑾黛" in the exemplars. Thereafter, the participants were asked to list no more than 10 features which they considered to be the most important in order to support their conclusion.

All features listed by the participants were counted, categorised and analysed. Firstly, the total of 768 features listed by the 109 participants could be categorised into 39 separate features. Secondly, occurrence frequencies of those

Figure 2: Details of the top 10 features.

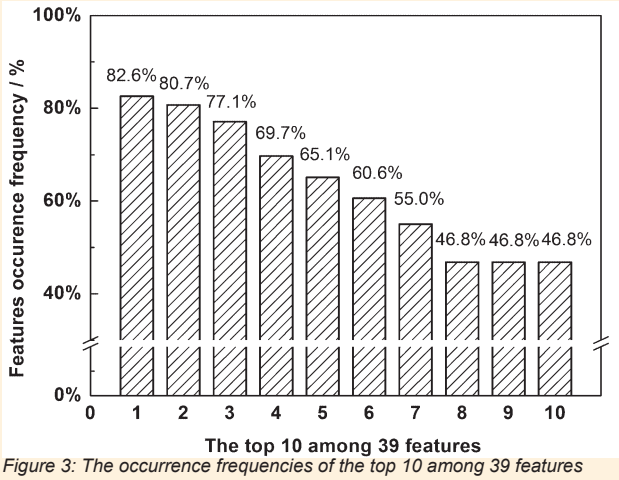

Figure 3: The occurrence frequencies of the top 10 among 39 features

39 separate features were compared. For example, Feature No. 1 (as shown in Table 1 and Figure 2) was mentioned by 90 participants. Its occurrence frequency was calculated as 90 divided by 109 to get the percentage of 82.6%. The value of this percentage was regarded as the standard of evaluating the significance for making the conclusion. Herein, this percentage is called the occurrence frequency. Thirdly, the 39 separate features were sorted in descending order of occurrence frequency. The top 10 features are illustrated in Figures 2 and 3, and explained in Table 1.

All 109 participants gave the correct identification conclusion, that is, the signature QD was written by the same person who wrote the exemplar signatures.

Results and Discussion

It is shown that stroke formation is the main characteristic for examiners to arrive at their conclusion from Figure 3 and Table 1. Among the top 10 features, seven were related to stroke formations. Furthermore, more than half of the examiners chose the top seven features, and nearly 50% of the examiners

No.	Features
1	Stroke formations of four dots in “黑”(black)
2	Stroke formations of the long “丿” in “廖”
3	Stroke formations of the first two strokes in “廖”
4	Overview features of the signature “廖瑾黛”
5	Stroke formations of three“丿” in “廖”
6	Proportion features between the first two strokes and the long “丿” in “廖”
7	Stroke formations of the“人” in “廖”
8	Stroke formations (pen lift) of “王” in “瑾”
9	Stroke formations of “廿” in “瑾”
10	Stroke sequence of the right part of “瑾”

Table 1: Details of the top 10 features.

chose the other three features. As most of the Chinese characters consist of multiple strokes, there are numerous stroke formations in writing a Chinese character. Therefore, examiners need to be able to identify significant features of stroke formations in order to arrive at the correct conclusion. In this study, the QD signature consists of 45 strokes making up three characters of which the structures are extremely complex, leading to more than 100 features to choose from according to the eight types of characteristics mentioned above. Therefore it is shown that stroke formation is a primary feature in complex Chinese signature examination.

Stroke formation can reflect writing actions such as pen lift, movement and pen pause, and its microscopic characteristics can reflect a person’s Chinese writing habits. It is also a notable distinction from letter-based signature identification such as the examination of English signatures, which use the line quality, fluency of writing, pen pressure variation, writing movement, and connection of strokes. The results of this study demonstrated that it is necessary for examiners to pay more attention to stroke formation in Chinese handwriting and signature examination, especially for those involving complex Chinese characters. The results also indicated that various comparison studies involving other types of writing such as simple signatures and cursive writing etc. should be designed in order to gather more useful information on the examination of various types of Chinese handwriting and signatures.

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The Forensic Importance of the Amelogenin Negative Male

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Abstract

The gender marker Amelogenin is commonly incorporated in commercial multiplex STR kits such as the AmpFISTR® Identifiler® PCR amplification kit. However, false gender determination can occur when there is dropout of the Amelogenin Y-allele, particularly in the absence of reference specimens. Failure of the Amelogenin sex test can result in misinterpretations and their forensic relevance should not be underestimated. This paper discussed the risks of failure of the Amelogenin sex test among the Indian and Malay ethnic groups in the Malaysian population. Its forensic relevance was demonstrated in a case study of an unknown Amelogenin negative male discovered in the DNA on a ladies decorative hair band.

Introduction

The gender marker provided in commercial STR kits such as the AmpFISTR® Identifiler® PCR amplification kits is based on the Amelogenin gene which is located on the X chromosome (Xp22.1-22.3) and the Y chromosome (Yp11.2) [1]. Sex determination is based on the detection of the two versions of the Amelogenin gene, which generally differ by a 6 bp deletion between the X and Y chromosomes [2]. The presence of both the X (106 bp) and Y (112 bp) copies in almost equal proportions indicates a male genotype while the presence of only the X copy indicates a female genotype. However, a failure to amplify the homologous Amelogenin part on the Y chromosome causes a dropout of the Amelogenin Y-allele and, as a consequence, the STR system falsely genotypes it as a female [3, 4].

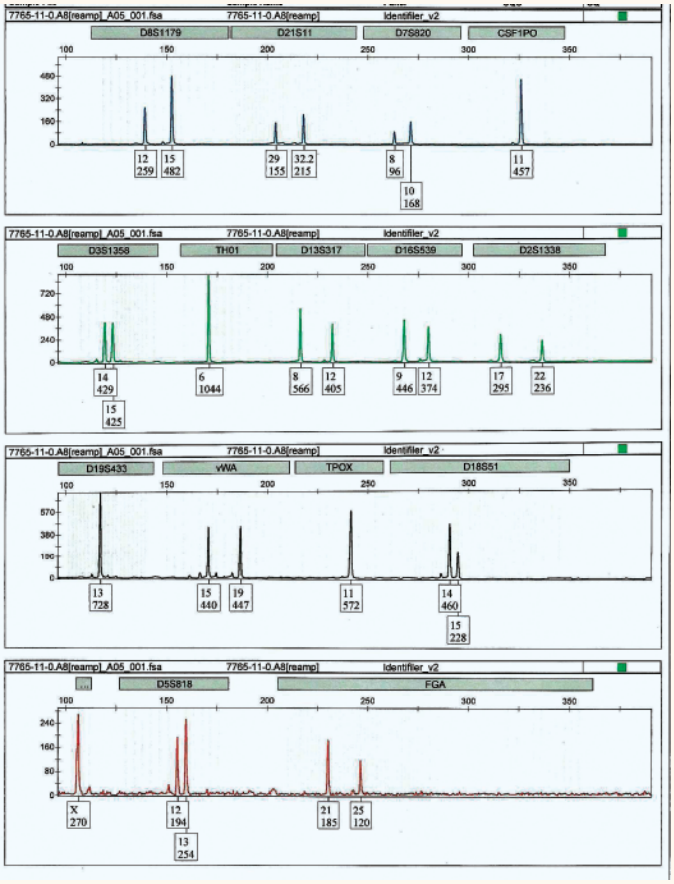

Figure 1: STR profile from DNA in a decorative hair band recovered from the crime scene of an Amelogenin negative male (conventionally genotyped as female).

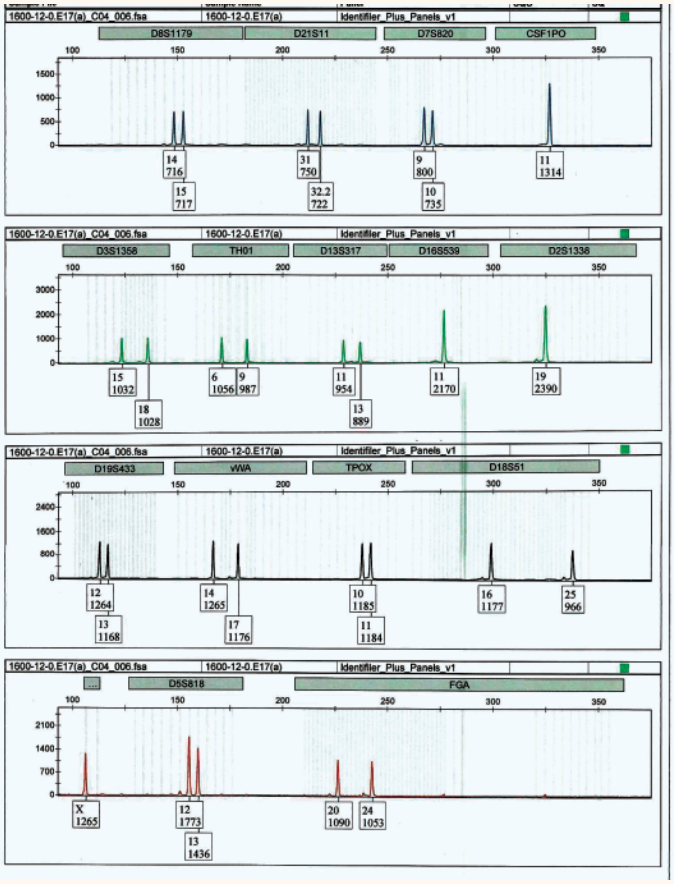

Figure 2: A typical STR profile from an Amelogenin negative male. Note the apparent lower peak height of the Amelogenin X allele in relation to alleles of the same colour dye.

Population studies on Malaysian male individuals revealed a significant proportion of Amelogenin Y-nulls in the Indian ethnic group (3.6% frequency) and 0.88% frequency in the Malay ethnic group due to a deletion of the gene on the Y chromosome and this deletion has been mapped to a region of at least 1.13 Mb on the Yp11.2 encompassing the Amelogenin, MSY1 minisatellite and DYS458 locus [5, 6].

Materials and Methods

Forensic casework specimens from a rape-and-kidnap case (suspects still at large) include a vaginal smear with detectable spermatozoa and a ladies decorative hair band recovered from the crime scene which was swabbed for contact DNA. DNA extractions were carried out by the phenol-chloroform method. DNA quantified by Quantifiler® indicated 0.2 ng/µl DNA yield in the swab from the hair band. PCR was carried out using the AmpFISTR® Identifiler® amplification kit. The amplicons were analysed on an Applied Biosystems 3130xl Genetic Analyzer using GeneMapper ID v3.2.1 software. In addition, Y-STR analysis was carried out using the AmpFISTR® Yfiler® amplification kit.

Results and Discussion

The DNA profile from the hair band genotyped as female (Figure 1) aroused no suspicion that it could be an Amelogenin Y-null profile. In a typical Amelogenin Y-null profile (Figure 2), the assessment of the Amelogenin X peak height (in relation to peaks of the same color dye) could be utilised in a balanced profile to predict Amelogenin Y-allele dropout. However, the stochastic effects evident in the profile of Figure 1 proved not helpful in elucidating this possibility. The absence of reference specimens from the suspecting individual(s) further confounded the discovery. The alert was raised when examination of the sperm extract from the vaginal smear (Figure 3) revealed the DNA types of Figure 1 as a major contributor. Y-STR profiling confirmed the contributor of the DNA in the hair band to be an Amelogenin negative male with allelic dropout at DYS458 (Figure 4), further confirming a deletion at the Y chromosome.

The encounter of the Amelogenin negative male in crime stains was not unusual in the Malaysian casework scenario as observed by the author and others [3]. The incidence of Amelogenin Y-nulls was reportedly significant in the Malaysian population among the Indian and Malay ethnic groups [3, 4]. This assumed importance and relevance in forensic casework. It cannot be overemphasised that the presence of the Amelogenin negative male is a serious consideration in crime stains genotyped as female as crime perpetrators have been generally acknowledged to be male prevalent.

Y-STR profiling is usually utilised to confirm an Amelogenin Y-null crime stain. In a recent initiative by the FBI CODIS (Combined DNA Index System) Core Loci Working Group to review and expand the current CODIS core loci, the Group recommended the inclusion of additional STR loci including the DYS391 locus to be added in a single amplification kit [7]. Addition of this one small Y-STR locus in an autosomal STR kit will confirm Amelogenin null values sometimes present in DNA typing by just one multiplex reaction. This is an approach to be lauded and will resolve the dilemma of false sex genotyping of the Amelogenin negative male.

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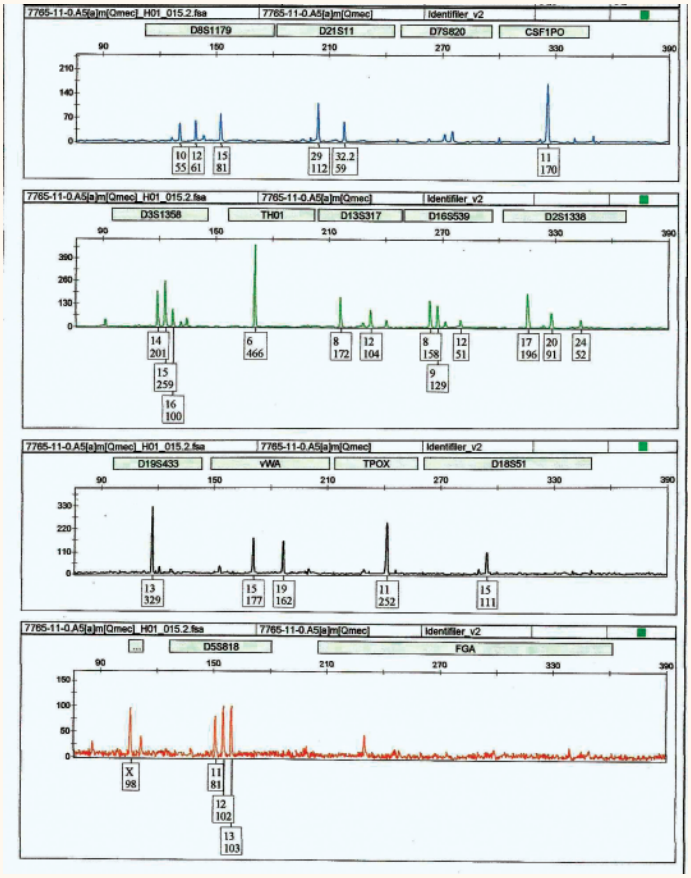

Figure 3: STR profile of sperm extract from vaginal smear consisting mainly of DNA types from Figure 1.

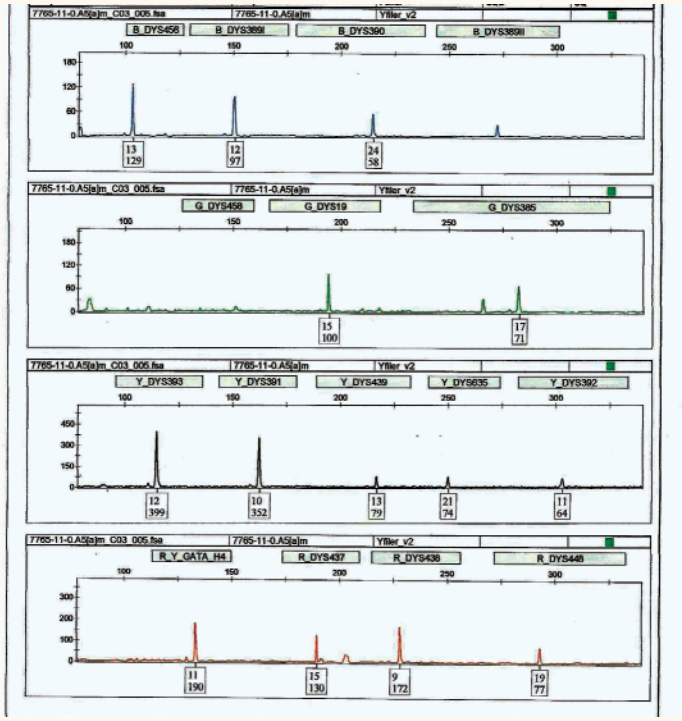

Figure 4: Y-STR profile of the Amelogenin negative male in Figure 1. Deletion of the DYS458 locus is observed.

Case Study

Determination of Hazardous Elements in a Poisoning Case by ICP-MS

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Introduction

Analysis of heavy metal poisons is an important part for forensic toxicologists. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a powerful tool to examine heavy metal poisons [1-3]. Typical heavy metal poisons are arsenic (As), mercury (Hg), lead (Pb) and thallium (Tl). In recent years, the use of compounds containing these heavy metals has decreased due to their toxicological and environmental impacts. However, in Chinese medicines, some of them are used as ingredients, such as vermilion (HgS). The intake amount of these medicines should be strictly controlled. In a case which happened in the rural area of northeastern China last year, a quack doctor applied a kind of poultice on the skin of a boy who had psoriasis. The boy had a fever and died 20 days later. In this case study, solution-based ICP-MS was employed for determination of As, Hg and Pb concentrations in the soft tissues including the heart, liver, kidney, subcutaneous tissue and heart-blood after microwave digestion of the samples.

Materials and Methods

Chemicals and standards

Standard solutions used for the ICP-MS measurement was ICP Multi Element Standard SolutionVI CertiPUR (Merck, Darmstadt, Germany) with 10 mg/l of lead, 100 mg/l of arsenic. In addition, 1000 mg/l single element mercury standard solution (Merck, Darmstadt, Germany) was used throughout the study to prepare standard solutions. Ultrapure nitric acid (65%, Merck, Darmstadt, Germany) and hydrogen peroxide (31%, Merck, Darmstadt, Germany) were used to make microwave digestion. Purified water (18.2 MΩ) from a Milli-Q water purification system (PURELAB, ELGA, UK) was used to prepare all the solutions.

Acid digestion

All the samples except blood were homogenised with purified water by the weight ratio of 1:1. Acid digestion of all the samples was carried out in a microwave digestion system (ETHOS A, Milestone, Italy) using 1.5 g of the homogenate, 6 ml of HNO3 and 2 ml of H2O2. Following the power program listed in Table 1, all the samples were decomposed at a maximum temperature of 200°C in sealed TFM tubes. After cooling, the samples were brought to 50 ml using purified water.

ICP-MS measurements

The measurement was carried out on ICP-MS (ThermoFisher, XseriesII, USA). The instrument was optimised daily with consideration of background count, sensitivity, as well as doubly charged ions and oxides formation. The quantitative calibration curves were built using yttrium (1 µg/l, for As) and terbium (1 µg/l, for Hg and Pb) as internal standards. Collision Cell Technology (CCT) was used to avoid polyatomic interference to arsenic measurement with H2-He (volume ratio of 3:97) as cell gas. The calibration curves for As, Hg and Pb showed linear relationship with correlation coefficients of 0.9998, 0.9995 and 0.9998, respectively.

Step	Time (min)	Temperature (°C)	Power (watt)
1	2	85	Up to 1000*
2	4	135	Up to 1000
3	5	200	Up to 1000
4	15	200	Up to 1000
5	Cooling time	Room temperature	0

*Note: depends on the number of the tubes

Table 1: Microwave digestion procedure.

	As	Hg	Pb
Blank blood (μg/ml)	0.0047	0.025	0.065
Heart tissue (μg/g)	3.97	0.209	0.258
Liver tissue (μg/g)	43.07	0.791	0.611
Kidney tissue (μg/g)	20.64	9.17	1.396
Heart-blood (μg/ml)	1.512	0.213	0.369
Subcutaneous tissue (μg/g)	6.55	4.45	29.66
Normal content in blood (μg/ml)	<0.03	<0.02	<0.05

Table 2: As, Hg and Pb content in samples and normal content in total blood.

Results and Discussion

ICP-MS measurements revealed elevated As, Hg and Pb concentrations in all investigated samples. The content of these three elements in samples (listed in Table 2) were up to hundred, even thousand, times of the normal content in total blood [4], indicating evidence for these heavy metal elements poisoning.

It is rare that these three heavy metals co-existed in one formula of Chinese medicine to cause the poisoning in this case. According to the content of the elements in different tissues, it is shown that the path by which the poisons entered the body was through the skin. These heavy metals were absorbed into the body and then redistributed in different tissues. Arsenic was deposited mainly in liver and kidney tissue, while mercury was deposited in kidney tissue. Lead was metabolised slowly and it appeared mainly in skin and subcutaneous tissues.

Conclusion

The formula of Chinese medicine is very complicated. In cases involving Chinese medicine, phytotoxin, animal toxin and heavy metals should be carefully considered. ICP-MS method is useful to examine heavy metal poisons, especially in soft tissues.

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Case Study of Identification of Buprofezin in Biological Samples by Gas Chromatography–Mass Spectrometry

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Introduction

Buprofezin (2-tert-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one) is a broad-spectrum insect growth regulator that interrupts the development of immature insects by inhibiting chitin biosynthesis and subsequent cuticle deposition (Figure 1) [1]. This compound has been widely used on tea, rice, potatoes, citrus fruit, cotton, and vegetables to control various pests, such as whiteflies, planthoppers, leafhoppers and scales [2].

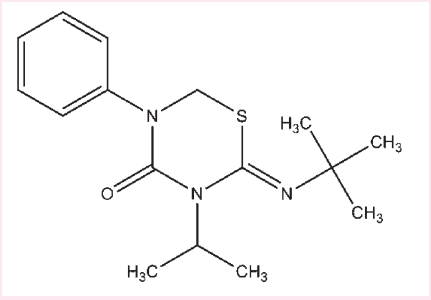

Figure 1: Buprofezin chemical structure.

Several methods have been published for the determination by gas chromatography and liquid chromatography of buprofezin in soils, fruits and vegetables [3]. No method has been published for the determination of this insecticide in human samples.

This study reports one case involving buprofezin poisoning. A gas chromatography-mass spectrometry (GC-MS) method was developed to detect and confirm this insecticide in post-mortem samples.

Case report

A nameless corpse with initial putrefaction was found in a construction site. No sign of violence was found on the body. At the scene, the local police reported that an empty plastic bottle was found.

The autopsy performed by local medical examiner was negative, and he could not specify the cause of death. Toxicological analyses were requested. Frozen samples of stomach, liver and the plastic bottle were taken for toxicological analysis.

Materials and Methods

Instrumentation

The analysis of buprofezin was performed on a Shimadzu GC-2010 system coupled with QP 2010 Plus mass spectrometer detector (Kyoto, Japan). A fused-silica capillary column (30 m×0.25 mm) coated with 0.25 μm bonded film of DB-5 (Agilent) was used. The GC column temperature program used was as follows: 100°C for 2 min, then ramped to 280°C at a rate of 30°C/min (held at this temperature for 17 min). The injection temperature was 280°C, and the injection volume was 1 μl made in the split mode (split ratio 10:1). The column flow was set at 1.00 ml/min. The mass spectrometer was operated in the electron impact mode with an ion source temperature of 210°C. The MS scanned mass range from m/z 40 to 550 was used for determination of the studied insecticide.

Chemicals and reagents

Chromatographic grade methanol was purchased from Merck (Darmstadt, Germany), and buprofezin standard was purchased from Dr. Ehrenstorfer (Germany). Stock standard solution was prepared in methanol at concentration of 1 mg/ml and stored at 4°C. Analytical standard solution was prepared from above stock standard solution.

Sample preparation

Control and calibration samples were prepared by spiking blended liver samples with standard solutions.

The plastic bottle taken from the scene was washed with 500 μl methanol, and diluted to 1000 times.

Homogenates of tissue samples (stomach and liver) was prepared by adding 1 g tissue to 1 ml distilled water and blended.

Liquid-liquid extraction was performed two times with 5 ml dichloromethane, and the tubes were agitated on a platform shaker for 10 min. After centrifugation at 3000 rpm for 10 min, the supernatant was evaporated to dryness under a slow stream of nitrogen at 50°C. The dried extracts were reconstituted with 200 µl methanol.

An aliquot (1 µl) of the above samples was injected into the GC/MS system.

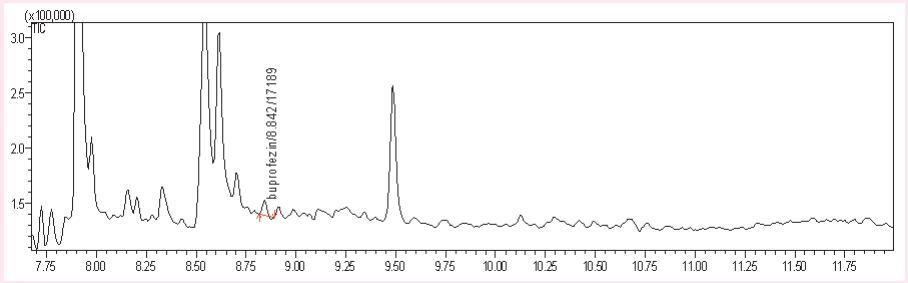

Figure 2: TIC chromatogram shown with the associated retention time in SCAN mode of buprofezin detected in the liver sample.

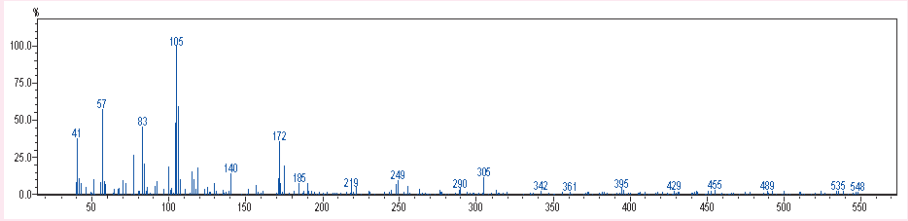
Figure 3: Mass spectrum of buprofezin detected in the liver sample.

Results and Discussion

Calibration curve for buprofezin in liver sample was linear from 0.2 to 20 µg/g (y = 382.4x + 16472 with r^2=0.9989, seven calibration points). The detection limit of buprofezin in liver sample was 0.05 µg/g (S/N=3) and the lower limit of quantification (S/N=10) was 0.2 µg/g. Analytical recovery was tested at the concentration levels of 0.5, 2 and 10 µg/g and determined by comparing the representative peak areas of buprofezin extracted from spiked blank liver with the peak area of a methanolic standard at the same concentration. The mean recovery was 85% with a coefficient of variation of 3.8%. For intra-day and inter-day precision determination, five replicate analyses were performed at three studied concentrations. The validation data for recovery were all above 75%. The method proved to be precise for buprofezin, both in terms of intra-day and inter-day analysis, with coefficients of variation (CV) less than 10%.

The procedure described proved to be sensitive and reproducible, and thus the developed method was applied to the fatal case presented. The GC/MS results showed the presence of buprofezin in the analysed samples. Gas chromatogram and mass spectrum of buprofezin detected in liver sample are shown separately in Figures 2 and 3.

Conclusions

This study presented a method for the determination of the insecticide buprofezin in biological samples by employing GC-MS analysis. The present GC-MS method has been successfully demonstrated to be accurate, precise and reproducible with advantages of being rapid and simple in one fatal case. To our knowledge, no published data reporting fatal cases due to buprofezin ingestion were found. It was the first time that a post-mortem tissue determination was performed for buprofezin.

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Comparison of Trace Paint and Plastic in a Traffic Accident Investigation

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Abstract

Three analytical methods, namely stereomicroscopy, fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS) were used to compare known and questioned paint and plastic samples in a traffic accident investigation. It is very important to distinguish between trace paint and trace plastic of similar colours in such cases.

Introduction

The transfer of paint and plastic occurs in a variety of cases from burglary to hit-and-run accidents, and analysis of motor vehicle paint and plastic is frequently performed in forensic work [1]. However, forensic paint samples are typically analysed in limited amounts that may preclude the application of standard paint analysis procedures or protocols. While smeared or abrasively transferred samples may provide more useful information, they are generally far more difficult to analyse [2].

This paper introduces the examination and comparison of paint and plastic traces on a tyre surface after a traffic accident. In this case, a motorcycle rider was seriously injured when his motorcycle allegedly collided with an overtaking high-power tractor. The tractor driver refused to acknowledge that a direct crash had occurred between the two vehicles.

Figure 1: (a) Photograph of the red motorcycle; (b) damage mark on the back cover; (c) the paint layers on a white polymeric substrate of the red motorcycle under magnification.

As damages and striations were found on the red paint surface of the motorcycle, a known red sample was collected from the motorcycle for comparison. Microscopic examination indicated that the red motorcycle metallic paint had a basecoat/clearcoat finish on a white polymeric substrate (Figure 1). On examination of the huge tyre of the tractor, questioned traces of some thin adhering mixture of red and white materials were found (Figure 2).

Figure 2: Photograph of the huge tyre (left) and adhering mixture of red and white under magnification (right).

Methods of Comparison

First, microscopic examination of physical features was carried out using a Leica MZ APO stereomicroscope. Next, FT-IR analysis using a Perkin Elmer Spectrum GX FT-IR with a diamond anvil cell (DAC) was conducted using the transmission mode, 32 scans at a resolution of 4 cm^{-1}, and a spectral range from 400 to 4000 cm^{-1}. Finally, SEM/EDS analysis was performed on a Philips XL30 ESEM and EDAX EDS. The working distance of SEM was set at 10 mm, and the accelerating voltage at 25 keV. The EDS was operated with a dead time of c. 30% and counting time of 50 s.

Results and Discussion

Under the stereomicroscope, the questioned traces on the tractor type appeared as a mixture of red material, twinkling tiny particles mingled with some tiny sand particles, and white semi-transparent materials. The red material had a similar colour and micro-texture as the known red motorcycle paint chip. The mixture of red material and the twinkling tiny particles was extracted together and analysed by FT-IR and was found to be metallic paint (designated as “Sample2-red”).

After several extractions and FT-IR analyses, the white semi-transparent materials were found to consist of two different chemical substances. The more easily separated and extracted component was a plastic (designated as “Sample2-plastic”). The other substance, which was difficult to separate and extract into its constituents due to the commingling of red paint and contaminating tiny sand particles, was paint (designated as “Sample2-white”).

The clearcoat, red basecoat layer and white polymeric substrate of the known motorcycle paint chip (designated as “Sample1-clear”, “Sample1-base” and “Sample1-poly”, respectively) were separated and extracted for FT-IR examination.

The IR spectra of Sample1-poly and Sample2-plastic both had the same absorption peaks at 967, 993, 912, 2237, 1602, 1583, 1494, 1452, 760, 700, 546 cm^{-1} (Figure 3a, 3b), and some minor differences (possibly due to contamination by sand particles), indicating that the chemical compositions of both of them were Acrylonitrile-Butadiene-Styrene (ABS).

The mixture of Sample2-white and Sample2-red (designated as “Sample2-mix”) was analysed by FT-IR since the IR spectra of the pure Sample2-white could not be obtained. The mixture of Sample1-clear and Sample1-base (designated as “Sample1-

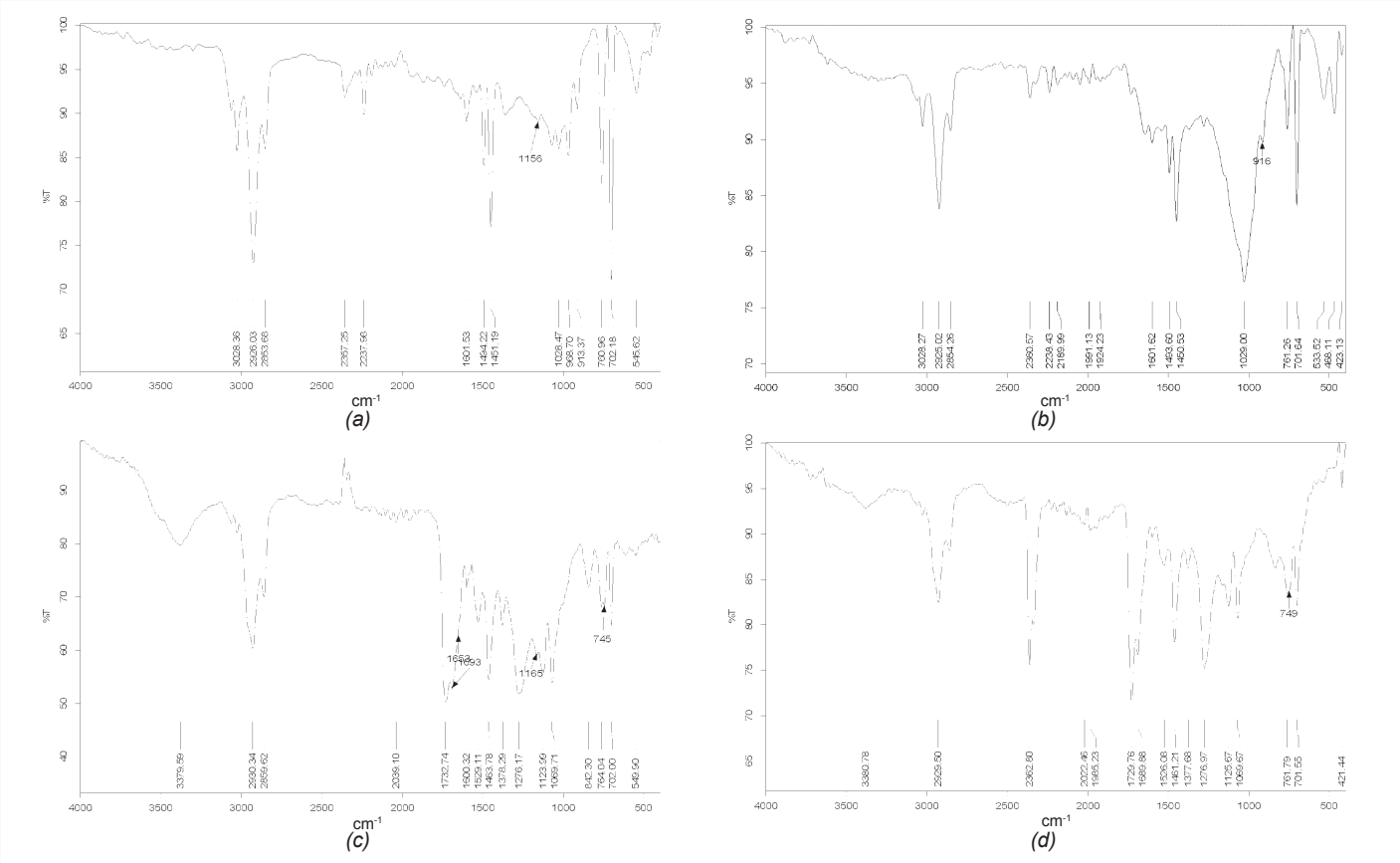

Figure 3: (a) The IR spectrum of Sample1-poly; (b) the IR spectrum of Sample2-plastic; (c) the IR spectrum of Sample1-mix; (d) the IR spectrum of Sample2-mix.

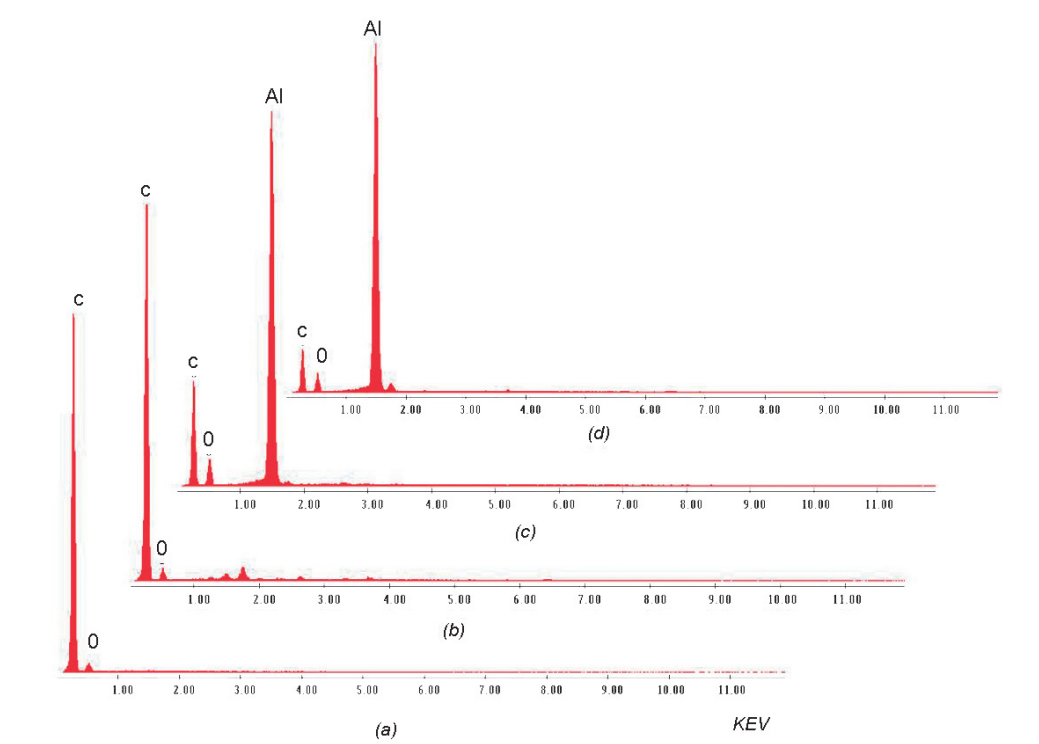

Figure 4: (a) The EDS spectrum of Sample1-poly; (b) the EDS spectrum of Sample2-plastic; (c) the EDS spectrum of Sample1-mix; (d) the EDS spectrum of Sample2-mix.

mix") was also analysed by FT-IR. The IR spectra of "Sample1-mix" and "Sample2-mix" both had absorption peaks at 1730, 1690, 1526, 1466, 1377, 765, 701, 1655, 1277, 1069, 842, 741 cm^{-1} (Figure 3c, 3d), indicating that both of them were mixtures of polyurethane and nitrocellulose paint.

On elemental composition analysis of the pigment by SEM/EDS, Al was unsurprising found in both Sample1-mix and Sample2-mix (Figure 4c, 4d). SEM/EDS analysis further revealed that both Sample1-poly and Sample2-plastic did not contain inorganic pigment (Figure 4a, 4b).

Conclusion

Three complementary methods were employed for comparing known red paint from a motorcycle and reddish smears on the tyre of a tractor suspected to have been involved in a traffic collision. Based on the above laboratory results, the following conclusions can be drawn: Sample1-poly and Sample2-plastic are the same class plastic, and Sample1-mix and Sample2-mix are the same class paint. Hence, the possibility that the trace mixture of red and white on the tyre surface have been transferred from the red motorcycle to the tractor tyre cannot be eliminated.

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DNA Test Leads to Reunion of Child with Real Mother

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This paper describes a case where a routine DNA paternity test requested by an alleged father revealed that his partner was not the child's biological mother. It shows how questioned maternity was resolved with the use of genetic evidence and highlights the importance of inter-agency cooperation in evaluating claims that are not consistent with scientific data.

The case was brought to the laboratory by a man whose girlfriend claimed that he was the father of her son. The laboratory ran a standard paternity test at 15 autosomal Short Tandem Repeat (aSTR) DNA markers using PowerPlex® 16 (Promega Corp., Madison WI). The DNA test showed that the man was not the biological father of the child.

Surprisingly, the profiles of the woman and the child did not match at two of the 15 aSTR DNA markers tested, namely D21S11 and D18S51, thus requiring careful scrutiny of the case (Table 1). The presence of one to two mismatches between a mother and her child may have resulted from rare mutational events that occurred at meiosis [1-3], hence a minimum of three non-matching markers are needed to exclude a putative parent as the true biological parent of a child [4, 5]. A second human identification kit, AmpFISTR® Identifiler® (Life Technologies, Carlsbad CA) was used to amplify the samples of the woman and child in order to include two additional DNA markers, D2S1338 and D19S433, that were not present in PowerPlex® 16 (Table 1). The second amplification increased the number of mismatching DNA markers from two to four, thereby excluding the woman from being the biological mother of the child.

Autosomal STR DNA Markers	Woman	Child
D21S11[a,b]	28, 29	30, 31
D18S51[a,b]	15, 18	16, 19
D2S1338[b]	18, 24	22, 23
D19S433[b]	14	13

[a]PowerPlex® 16; [b]AmpFISTR® Identifiler®

Table 1: Autosomal STR DNA profiles of the woman and child at the four excluding markers.

In many countries, the child's welfare is paramount. Since scientific results already showed that the woman who had custody of the child was not the child's mother, this was a potential case of child-trafficking. When presented with the results of the test, the man was concerned as to the identity of the child's biological parents. He requested that the Department of Social Welfare and Development (DSWD) be appraised of the situation.

Immediate attention was given to the case due to the Memorandum of Agreement (MOA) between the DSWD and the University of the Philippines (UP) in connection with the DNA-ProKids program (www.dna-prokids.org). This international humanitarian initiative aims to use DNA in deterring the trafficking of children. The laboratory conducted additional tests in order to verify if the woman and the child are maternally related. This was done through sequencing of the mitochondrial hypervariable regions I and II (mtDNA HVI, HVII) of both samples. The mtDNA sequences of both samples were identical hence, the woman and the child are related maternally [6]. Subsequent onsite investigation by a DSWD social worker revealed that the woman was in fact the child's maternal grandmother. She had taken her own daughter's child to obtain financial support from her boyfriend since her daughter was not financially stable. After counseling with the DSWD social worker, the woman decided to return the child to his true parents.

This case highlights the importance of DNA in reuniting children with their biological parents, the value of using a scientific approach in forensic investigations, and the advantage of partnerships between academic institutions and government agencies in providing service to the public.

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(As at 1 November 2012)