ForensicAsia

THE ASIAN FORENSIC SCIENCES NETWORK NEWSLETTER | ISSUE 15 | SEPTEMBER 2025

AFSN President's Address

To All Esteemed Members of the Asian Forensic Sciences Network (AFSN),

Dear colleagues and friends,

Greetings to all colleagues in Asia and around the world!

With great pleasure, I would like to express my heartfelt appreciation to all the Asian Forensic Sciences Network (AFSN) members as we embark on the latest issue of our publication. This platform acts as a testament of vibrant spirit on scientific collaboration, not only as a record of our shared accomplishments, but also as a reflection of the unity and dedication that continues to shape the growth and impact of our beloved Network.

AFSN has gone through tremendous journey since its first publication in 2009, and achieved distinguished milestones across the technical, academic, and institutional spheres, reflecting its unwavering pursuit of excellence. From inter-laboratory collaborations to regional training programmes and capacity-building workshops, the dedication and perseverance demonstrated by our AFSN members in advancing forensic standards and practices are truly commendable.

As we look ahead to the 17th AFSN Annual Meeting and Symposium in 2025, to be convened in Incheon, Republic of Korea, I wish to express my profound gratitude to the National Forensic Service (NFS) Republic of Korea for their distinguished support in hosting this landmark event. We look forward with great anticipation to an intellectually stimulating convergence, one that will not only foster the exchange of pioneering ideas and scientific insights but also to reinforce our shared commitment to continual advancement of forensic science through purposeful collaboration and collective vision.

Recent noteworthy developments is the interim formation of the new Traffic Accident Reconstruction Workgroup, under the leadership of Dr. Kim Jonghyuk from NFS. This forward-looking initiative underscores the dynamic evolution of forensic science and the growing imperative for specialised expertise in vehicular forensics and collision analysis. A warm invitation to all interested professionals to engage with and contribute to the continued advancement of this essential domain.

We are equally proud to announce the appointment of a newly constituted panel of Guest Editors for *ForensicAsia.* These esteemed experts representing a broad spectrum of forensic disciplines and bringing with them extensive regional and international experience will be instrumental in enhancing the journal's academic rigour and elevating its scientific standing within the global forensic community.

With the recent addition of new member institutes, our Network has now expanded its reach across 19 countries, a testament to the growing influence and relevance of AFSN in the regional forensic landscape. To our newly accepted member institutes, I extend a heartfelt and enthusiastic welcome. Your inclusion marks the beginning of a promising new chapter for AFSN, and we look forward to your active engagement and valuable contributions as we collectively strive to advance forensic science across Asia and beyond.

AFSN members are strongly encouraged to actively cultivate inter-workgroup collaboration. The complexities of contemporary forensic challenges demand inherently multidisciplinary solutions, bridging digital, chemical, biological, and reconstruction sciences. By fostering deeper synergy across our Workgroups, we can collectively advance more comprehensive, innovative, and impactful forensic outcomes.

We also take this opportunity to acknowledge the vital contributions of our member institutes across the region. Though much of your work occurs behind the scenes, its impact is both profound and indispensable. Your unwavering commitment to scientific integrity adoption of international best practices, and dedication to cross-border collaboration have strengthened justice systems and enhanced public safety throughout Asia. These efforts symbolize the spirit of excellence and cooperation that define the AFSN community and continue to inspire progress across the region and beyond.

During my recent visit to Uzbekistan for the Tashkent Law Spring 2025, I had the privilege of introducing the mission and impact of AFSN to an international audience, reaffirming our commitment to fostering dialogue and collaboration across legal and forensic communities. As we continue to grow, AFSN must strengthen its role not only as a regional pillar but as a global platform, deepening partnerships with other forensic networks and allied institutions. Enhanced international cooperation will amplify our cohesive voice, promote the harmonisation of standards, and uphold our shared dedication to scientific excellence, truth, and justice.

Let us move forward with a united vision, anchored in integrity, inspired by curiosity, and committed to excellence. The landscape of forensic science is evolving rapidly, and as forensic professionals, we bear the critical responsibility of navigating this complexity while safeguarding the principles of justice in an ever-changing world.

I extend my sincere gratitude to the editorial board of *ForensicAsia* for their steadfast commitment and exemplary efforts in ensuring the timely and high-calibre publication of this issue. Their diligent work, often undertaken behind the scenes plays a pivotal role in upholding the scholarly integrity and influence of our shared scientific platform.

To all our members, past, present, and new, thank you for your enduring support, your passion for forensic science, and your contributions to the unified strength of AFSN.

Congratulations once again, and my very best wishes for the continued success of our Network and its members. Happy reading!

TS. CHM NOR AIDORA SAEDON
AFSN President
Department of Chemistry, Malaysia

Editor's Address

Dear colleagues and members of AFSN,

Thanks to all our colleagues who have contributed their research and studies in this Issue 15 of *ForensicAsia*.

ForensicAsia has been an important newsletter which connects all our member institutes in the Asian Forensic Sciences Network (AFSN) since the inception of AFSN in 2008. I am so glad to see that we have your full support all along over the years.

In this Issue, we have an overwhelming number of 18 technical articles plus case studies, including, fingerprints, firearms, fires & explosions, forensic biology & DNA, illicit drugs, traffic accidents, toxicology, forensic linguistics, forensic medicine, quality assurance and general forensic science. In addition, we have also received contributions on AFSN news, members' news, and introduction of our new member institutes.

I would like to take this opportunity to thank all the authors who have contributed to the 15th Issue of *ForensicAsia.* I would also like to thank our guest editors who have spent their time in reviewing the articles, and our editorial assistants who have helped in the administrative matters and put together articles and artwork of this publication, to make it a success.

Happy reading and see you in Incheon!

Dr Lui Chi Pang
Editor

Content

Content

For enquiries, feedback or contribution of articles, please email to ***asianforensic@outlook.com***

For contribution of articles, please refer to the guidelines at ***www.afsn.asia***

A Forensic Medicine Workgroup Activity: Bring Your Own Case Forum

Assoc. Prof. Wisarn Worasuwannarak, M.D.
Chairperson of the Forensic Medicine Workgroup
Forensic Medicine Laboratory, Department of Pathology,
Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand

Summary

Dr. Wisarn Worasuwannarak, chairperson of the Forensic Medicine Workgroup, inaugurated the "Bring Your Own Case Forum" webinar series, planned to occur three times annually, with hosting responsibilities among Thailand, China, and the Philippines. The inaugural session featured three expert presentations addressing diverse and complex forensic cases.

The first presentation, delivered by Dr. Gorn from Thailand, explored a case involving a young female with multiple stab wounds, raising critical questions about whether the manner of death was suicide or homicide. Dr. Benjamin from Singapore followed with a detailed analysis of a sodium nitrite toxicity case, underscoring the challenges of detecting methemoglobin and the alarming rise in intentional sodium nitrite ingestion. Finally, Dr. Nola from Indonesia presented two cases of subcapsular hemorrhage of the liver, potentially linked to cardiopulmonary resuscitation (CPR), highlighting the importance of recognizing CPR-related injuries during forensic examinations.

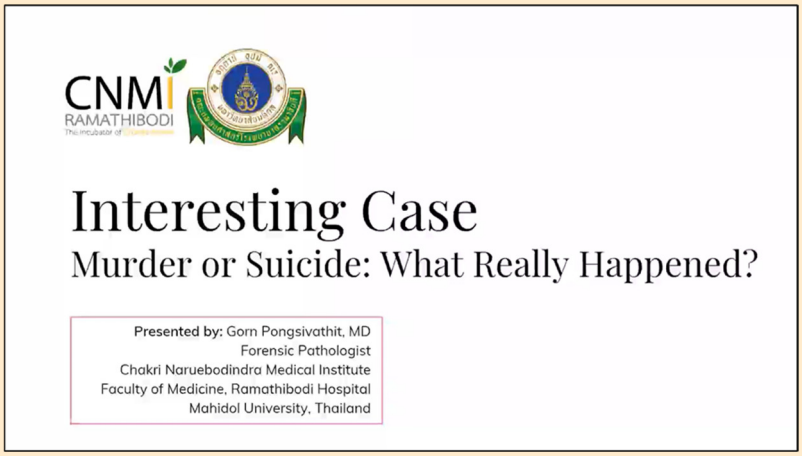
The forum concluded with discussions about future initiatives, including preparations for the AFSN 2025 conference, which is set to take place in Seoul, South Korea. These cases and discussions reflect the forum's commitment to fostering international collaboration and advancing expertise in forensic medicine.

Introduction and Overview of the Forum

Dr. Wisarn Worasuwannarak, the chairperson of the Forensic Medicine Workgroup (FMWG), introduced the "Bring Your Own Case Forum" and highlighted its purpose as an international inter-hospital conference for forensic doctors. This forum serves as a platform for knowledge exchange, collaboration, and sharing of unique case studies among professionals in forensic medicine. Dr. Wisarn announced plans to hold the forum three times a year, with Thailand, China, and the Philippines designated as hosts for the upcoming sessions.

Additionally, Dr. Wisarn emphasized the potential for exchange programs that would allow forensic professionals to study and experience forensic medicine systems across Asian countries, fostering regional expertise and cooperation. He also outlined the workgroup's long-term goal of participating in the AFSN 2025 conference, scheduled to be held in South Korea in September 2025. These initiatives underscore the commitment of the FMWG to advancing forensic medicine through collaboration and international engagement.

First Case Presentation: The Murder or Suicide, What Really Happened?



Dr. Yudy, the secretary of the Forensic Medicine Workgroup (FMWG), introduced the first presenter, Dr. Gorn Pongsivasathit from Thailand, providing an overview of his educational qualifications and current professional role. Dr. Gorn commenced his presentation, titled **"Murder or Suicide: What Really Happened?"**, by sharing detailed case information.

The case centered on a young adult female discovered with multiple stab wounds to the chest and neck, accompanied by an implanted cardiac device (ICD), raising critical questions regarding the manner of death. Dr. Gorn provided a comprehensive analysis of the autopsy findings, which revealed a significant hemothorax in both pleural cavities. He also addressed the initial inquiries from law enforcement, which sought clarity on whether the death resulted from homicide or suicide. This case highlighted the intricate interplay of forensic investigation and medico-legal interpretation in determining the circumstances surrounding an individual's death.

Discussion on the Stab Wounds and Manner of Death

During the discussion, participants analyzed the case, particularly focusing on the multiple stab wounds to the chest and the absence of defensive injuries. Dr. Gorn elaborated on the police's initial suspicion of homicide, which prompted an investigation that included scrutiny of the deceased's boyfriend.

The discourse also considered the possibility that some of the wounds might represent hesitation marks, potentially pointing toward suicide as the manner of death. In his concluding remarks, Dr. Gorn shared the final opinion provided to law enforcement, which suggested suicide as a plausible explanation but emphasized the necessity of continued investigation to confirm the findings. This collaborative discussion underscored the complexities involved in distinguishing between homicide and suicide in forensic cases.

Second Case Presentation: Sodium Nitrite Ingestion in Singapore

Dr. Yudy introduced the second presenter, Dr. Kuek Jia Wei, Benjamin, from Singapore, providing an overview of his educational achievements and current professional role. Dr. Benjamin presented a case involving a 16-year-old Filipino male who succumbed to sodium nitrite toxicity following intentional ingestion.

The case included key findings such as a suicide note, a glass cup containing whitish residue, and two bottles labeled sodium nitrite, one of which had been opened. Dr. Benjamin detailed the autopsy findings,

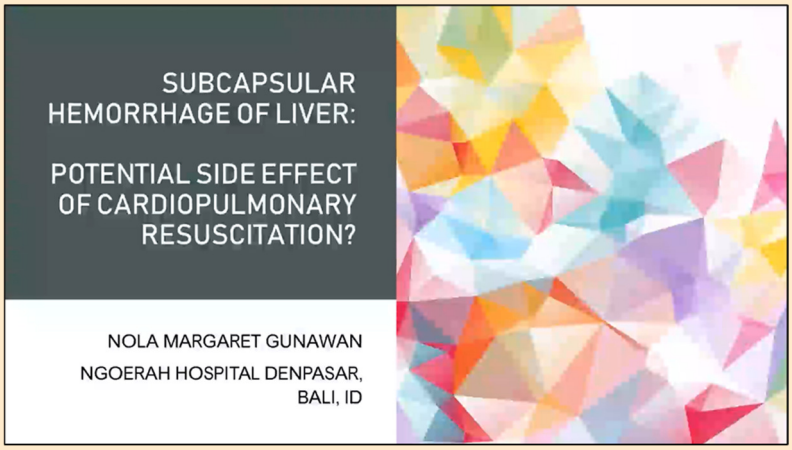
which revealed a distinct brownish discoloration of the hypostasis and internal organs. Laboratory analysis confirmed the presence of nitrite methemoglobin in the post-mortem blood and urine, consistent with sodium nitrite toxicity. This case emphasized the forensic challenges associated with detecting methemoglobin and highlighted the rising trend of intentional sodium nitrite ingestion in suicide cases.

Discussion on Sodium Nitrite Toxicity and Its Implications

Dr. Benjamin elaborated on the physiological effects of sodium nitrite on the body, highlighting its role in the formation of methemoglobin and nitric oxide. These compounds interfere with oxygen transport and utilization, resulting in tissue hypoxia and vasodilatory shock, which can be fatal.

He emphasized that diagnosing sodium nitrite toxicity requires a high index of suspicion and specialized testing to identify nitrites and quantify methemoglobin levels. Dr. Benjamin also discussed the challenges associated with accurately measuring methemoglobin, noting the occasional reliance on a colorimetric chart as a rapid, albeit approximate, diagnostic tool. The presentation underscored the growing prevalence of intentional sodium nitrite ingestion, often facilitated by online availability, and stressed the need for increased awareness and preparedness among forensic pathologists to address this emerging trend effectively.

Third Case Presentation: Subcapsular Hemorrhage of the Liver Due to CPR



Dr. Yudy introduced the third presenter, Dr. Nola Margaret Gunavan from Indonesia, highlighting her educational qualifications and current professional position. Dr. Nola presented two compelling case reports examining subcapsular hemorrhage of the liver, potentially caused by cardiopulmonary resuscitation (CPR).

The first case involved a 29-year-old Chinese female found floating in the sea, with a documented history of CPR administered by a medical professional. The second case concerned a 50-year-old Chinese female who was involved in a motor vehicle accident, with CPR performed by a tourist at the scene. In both cases, autopsy findings revealed subcapsular hemorrhages on the liver, alongside other injuries.

Dr. Nola elaborated on the forensic challenges of distinguishing between liver injuries caused by CPR and those resulting from other mechanisms, such as trauma. Her analysis emphasized the importance of correlating autopsy findings with the clinical and situational history to accurately interpret such injuries. These case studies highlighted the need for forensic pathologists to remain vigilant in assessing the potential impacts of CPR on internal organs.

Discussion on CPR-Related Injuries and Their Implications

During the discussion, participants examined the potential complications associated with cardiopulmonary resuscitation (CPR), including subcapsular hemorrhage of the liver and injuries to other internal organs. Dr. Nola emphasized the critical importance of differentiating between injuries caused by CPR and those resulting from external trauma, as this distinction is vital for accurate forensic analysis.

The conversation also addressed the impact of chest compression depth during CPR, noting its potential to contribute to internal organ injuries. Participants shared their professional experiences with cases involving CPR-related injuries, underscoring the importance of awareness among clinicians performing CPR. The discussion highlighted the need for balancing effective life-saving measures with minimizing the risk of injury to internal organs during resuscitative efforts.

Closing Remarks and Future Plans

Dr. Wisarn Worasuwannarak expressed his gratitude to the presenters and participants for their insightful contributions and active engagement throughout the forum. Following the conclusion of the presentations, Dr. Sohyung Park from South Korea provided details about the upcoming AFSN 2025 Conference, scheduled to take place in Seoul from 15 to19 September 2025.

Participants voiced their interest in attending the conference and discussed the potential for fostering international collaboration and establishing exchange programs to enhance forensic medicine practices across borders. The meeting concluded with a reminder for attendees to complete the certification form and a final note of appreciation to all participants for their valuable involvement in the forum.

Introduction to Identification Bureau, Hong Kong Police Force

Chief Inspector Chan Shuk-yee Tracy
Identification Bureau, Hong Kong Police Force,
Hong Kong SAR, People's Republic of China

As a specialist bureau under the Crime Wing of the Hong Kong Police Force, the Identification Bureau (IB) headed by a Senior Superintendent of Police provides professional forensic services to HK's law enforcement agencies in fingerprint examination, DNA sampling, firearms examination, and forensic photography. Joining the Asian Forensic Sciences Network (AFSN), IB is eager to connect with regional partners and contribute to the shared pursuit of forensic excellence.

The operations of IB are primarily divided into two forensic disciplines: fingerprint examination and firearms examination. The Forensic Firearms Examination Division is instrumental in examining arms and ammunition in criminal investigations, conducting on-site examinations of firearm discharge incidents, and providing expert testimony in judicial proceedings.

In the area of fingerprint examination, IB comprises five specialized sections:

1) ***Advanced Technology Section*** – An ISO/IEC 17025 accredited laboratory specializing in the development of latent fingerprints using scientific methods, conducting research on fingerprint recovery techniques, and supporting major crime scene investigations.

2) ***Scenes of Crime Section*** – An ISO/IEC 17020 accredited crime scene unit specializing in the on-site detection of latent fingerprints and the collection of DNA trace evidence.

3) ***Computer Assisted Fingerprint Identification System Section*** – Operates the computerized fingerprint identification system, performs both latent and known print comparisons, and maintains fingerprint records of convicted individuals.

4) ***Support Section*** – Performs manual fingerprint comparisons and prepares fingerprint evidence for court purpose.

5) ***Photographic Section*** – Provides crime scene and forensic photography services

IB officers provided professional forensic examination at crime scene

Crime Scene Investigation (CSI) Mobile Laboratory equipped with advanced equipment for latent fingerprints recovery at scene

To further enhance its operational capabilities, IB launched its first Crime Scene Investigation (CSI) Mobile Laboratory in December 2024. This purpose-built, mobile forensic laboratory is equipped with state-of-the-art tools and facilities, significantly improving the efficiency and responsiveness of the aforementioned forensic teams especially in complex crime scene examinations.

Looking ahead, IB is dedicated to work closely with AFSN partners, sharing expertise and contributing to the collective development of forensic science in Asia.

Scene of Crime Officer conducted fingerprint examination at crime scene

An Overview of the Office of Police Forensic Science, Royal Thai Police

Pol.Lt.Col. Sorada Pitilertpanya, Pol.Maj.Gen. Watee Asawutmangkul
Office of Police Forensic Science, Royal Thai Police,
Thailand

Introduction

The Office of Police Forensic Science is a forensic institution in Thailand under the administrative of the Royal Thai Police, playing a vital role in crime investigation, evidence analysis, and law enforcement support. Operating under internationally recognized standards, including ISO/IEC 17020 for crime scene investigation and ISO/IEC 17025 for forensic laboratory accreditation, the division upholds the integrity of forensic science within the Thai justice system.

As an integral part of the Royal Thai Police, the division employs modern forensic methodologies, advanced laboratory techniques, and cutting-edge digital forensic innovations. Its contributions extend beyond national borders, supporting judicial bodies and fostering international forensic collaborations.

Organizational Structure

The Office of Police Forensic Science is comprised of multiple specialized agencies, each dedicated to a distinct area of forensic investigation:

- ***Central Police Forensic Science Division*** – The principal forensic unit responsible for nationwide forensic operations and coordination.
- ***Police Forensic Science Center 1-10*** – Regional forensic units conducting crime scene investigations and evidence analysis across Thailand.
- ***Institute of Forensic Training and Research*** – A facility providing specialized training programs for forensic scientists, crime scene investigators, and law enforcement officers.
- ***Criminal Records Division*** – Maintains Thailand's national criminal database, including fingerprint records and forensic case archives.
- ***Explosives Information Center*** – Specializes in the forensic examination of explosive materials, devices, and residues related to terrorism and criminal activities.

Central Police Forensic Science Division Sub-Divisions

Each sub-division within the Central Police Forensic Science Division specializes in a key forensic discipline:

Crime Scene Investigation (CSI) Sub-Division

- Uses PolyLight enhancement and chemical processing to visualize latent fingerprints and biological evidence.
- Employs super glue fuming and digital imaging for evidence preservation.
- Utilizes 3D scanning and scene mapping for detailed crime scene reconstruction.
- Ensures systematic crime scene management for proper forensic material collection and storage.

Document Verification Sub-Division

- Conducts handwriting and signature analysis for fraud detection.
- Authenticates government-issued documents, banknotes, and securities to prevent counterfeiting.
- Uses forensic imaging techniques to detect alterations, erasures, and hidden modifications.

Firearm and Ammunition Identification Sub-Division

- Conducts microscopic comparisons of spent bullets and casings to identify firearms.
- Performs gunshot residue (GSR) analysis to determine firearm discharge.
- Carries out trajectory reconstruction and impact analysis for forensic ballistics investigations.

Latent Fingerprint Sub-Division

- Enhances latent fingerprints using dusting powders and cyanoacrylate fuming.
- Utilizes digital imaging and biometric analysis for fingerprint comparison.
- Expands forensic coverage to palm print and sole print identification.

Biology and DNA Sub-Division

- Identifies biological samples such as blood, semen, saliva, and hair.
- Conducts DNA extraction, amplification, and profiling using PCR and STR analysis.
- Performs comparative DNA analysis for suspect identification and kinship verification.
- Specializes in forensic casework for mass disaster victim identification (DVI) and criminal investigations.

International Collaboration and Forensic Standards

The Office of Police Forensic Science actively engages in international forensic cooperation, contributing to global forensic advancements. It collaborates with:

- **INTERPOL and ASEAN forensic networks** to exchange forensic intelligence with international countries.
- **Global forensic institutions** to enhance research, training, and casework methodologies.
- **International legal and law enforcement bodies** to facilitate forensic evidence exchange in transnational crime investigations.

Conclusion

The Office of Police Forensic Science stands as a pillar of forensic excellence in Southeast Asia. Through its specialized sub-divisions, adherence to international accreditation standards, and active participation in global forensic initiatives, the division continues to strengthen Thailand's forensic capabilities. By leveraging scientific expertise, technological innovation, and investigative support, it plays a crucial role in enhancing criminal investigations and upholding justice.

The First Forensic Science High-Quality Development Forum Focuses on Emerging Fields to Chart the Course for Industry Advancement

Prof. Di Peng, Dr. Li Shuo
Criminal investigation school, Southwest university of political science and law, People's Republic of China

To promote the high-quality development of China's Forensic Science industry and fully leverage the leading role of higher education institutions in industry advancement and talent cultivation, while commemorating the 20th anniversary of the promulgation of the '228 Decision' and advancing the legislative process of the Forensic Science, the "First Forensic Science High-Quality Development Forum & Academic Symposium on the 20th Anniversary of the '228 Decision'" grandly opened on the morning of 27 February 2025, at the Yubei Campus of Southwest University of Political Science and Law (SWUPL).

The symposium was hosted by SWUPL and Chongqing Municipal Justice Bureau, organized by SWUPL Forensic Science Center, School of Criminal Investigation, Engineering Research Center for Smart Justice (under cultivation), Legislative Research Institute of SWUPL, and Chongqing Forensic Science Association, with co-organization by the Journal of Southwest University of Political Science & Law and the Chinese Journal of Forensic Sciences. Nearly 250 participants attended the event, including officials in charge of Forensic Science work from judicial departments and associations across 31 provincial-level regions, representatives from 23 universities nationwide, and practitioners from Forensic Science institutions.

It is reported that the "Decision of the Standing Committee of the National People's Congress on Issues Concerning the Administration of Forensic Science" (hereafter referred to as the "Decision"), promulgated on 28 February 2005, has driven significant progress in China's Forensic Science sector over its 20-year implementation. In September 2023, the 14th NPC Standing Committee included the Forensic Science in its Category II legislative planning projects, marking a new developmental milestone for the industry.

During the keynote session chaired by Professor He Jiahong from Renmin University of China, Chen Zhiyuan, Member of the Party Committee and Deputy Director of Chongqing Municipal Justice Bureau, delivered a report titled "Reflections on Reform and Development Issues in Forensic Science Work." Professor Du Zhichun from East China University of Political Science and Law presented on "Exploring the Forensic Science System with Chinese Characteristics," while Professor Guo Hua from Central University of Finance and Economics addressed "The Fundamental Logic Behind Formulating the Forensic Science Law". Professor Chen Ruchao of Southwest University of Political Science and Law shared insights on "Legislating for Forensic Science: The Logic and Structural Framework of the Forensic Science Law".

Spanning two days, the conference featured synchronized academic discussions across a main venue and parallel sessions, structured around eight core thematic units. The main venue focused on Forensic Science theory and practice, while the parallel sessions hosted specialized forums: the "Frontiers of Scientific and Technological

Applications in Forensic Science" showcasing AI and other emerging technologies in the field, and the "Forensic Science Emerging Scholars Forum" highlighting research by young academics. Each session included expert presentations followed by vibrant debates among speakers and discussants.

The conference received over 180 papers addressing Forensic Science topics, categorized into four major themes: legal system refinement, management innovation, technological advancement, and talent development. After rigorous expert review, 118 papers were selected for inclusion in the *Proceedings of the First Forensic Science High-Quality Development Forum* (41 on legal system refinement, 36 on management innovation, 29 on technological advancement, and 12 on talent development).

This event not only established a high-caliber, multidisciplinary academic exchange platform for the Forensic Science industry but also exemplified our university's commitment to integrating "New Liberal Arts + New Engineering" disciplines. By fostering a collaborative "government-industry-university-research-application" innovation ecosystem, it advances the industry's high-quality development. Moving forward, our institution will continue driving technological innovation and capacity-building in Forensic Science, injecting sustained momentum into modernizing the Forensic Science system and advancing the rule of law in China.

Philippines Launches Ambitious Forensic Science Initiative in Landmark Collaboration

Dr. Maria Corazon A. De Ungria, PhD
DNA Analysis Laboratory, Natural Sciences Research Institute,
University of the Philippines Diliman, Philippines

Marking the 75th Anniversary of the Universal Declaration of Human Rights, the Philippines, represented by Executive Secretary Lucas Bersamin, has proudly announced an ambitious new goal: the establishment of a groundbreaking Training Institute for Forensic Science and Investigation by 31 December 2029. This landmark initiative aims to transform and elevate the nation's forensic investigative capabilities, aligning them rigorously with the internationally respected Minnesota Protocol standards.

Significantly boosted by international collaboration, this initiative has secured strong support from the Australian Embassy, which facilitated visits from esteemed forensic specialists Dr. Stephen Cordner and Dr. Richard Bassed of the Victorian Institute of Forensic Medicine and Monash University, and Justice John Champion of the Victorian Supreme Court. These international experts visited Manila in August 2024 to actively engage with key Philippine institutions, notably the University of the Philippines (UP).

As a result of these crucial discussions, a landmark Memorandum of Understanding was signed on 24 October 2024, between UP Manila Chancellor Dr. Michael Tee and Professor Craig Jeffrey, Deputy Vice-Chancellor (International) and Senior Vice-President of Monash University. This significant collaboration will offer advanced forensic training programs and innovative research opportunities, including specialized short-term courses and a comprehensive two-year Master's Degree in Forensic Medicine. Key areas covered will include forensic anthropology, forensic genetics/genomics, forensic chemistry, and forensic dentistry.

Leading this important educational advancement, Dr. Raquel Fortun, Chair of UP Manila's Pathology Department, has been appointed to head a specialized technical working group established by the Philippine Commission on Higher Education (CHED). The group's primary objective is to standardize and enhance forensic science curricula nationwide. Complementing this effort, the DNA Analysis Laboratory at UP Diliman, an active member of the Asian Forensic Sciences Network (AFSN), under the leadership of Dr. Maria Corazon A. De Ungria, has formally accepted an invitation to contribute significantly to the group's activities.

Simultaneously, Justice Champion is actively engaging with the UP College of Law, advocating the integration of advanced forensic methodologies into the legal curriculum. This collaboration emphasizes the critical importance of equipping legal professionals with cutting-edge forensic science knowledge to enhance the effectiveness and fairness of the criminal justice system.

Further affirming the nation's dedication to forensic science advancement, President Ferdinand Marcos Jr. signed Administrative Order 29 on 22 January 2025. This order established a dedicated technical working group responsible for developing the National Forensic Institute, comprising distinguished UP experts alongside senior officials from the Department of Interior and Local Government, Department of Foreign Affairs, Department of Health, Department of Budget and Management, and CHED.

The initiative formally began with notable enthusiasm on 17 March 2025, when Drs. Cordner and Bassed delivered their inaugural lectures at UP Manila, marking the start of an exciting and transformative new era in Philippine forensic science.

Introduction of the Shahe Technical Area of IFS, China

Mr. Zou Bo, Mrs. Qiao Ting, Mrs. Lin Min, Mr. Meng Qingzhen
Institute of Forensic Science, People's Republic of China

The Shahe Technical Area of Institute of Forensic Science (IFS) is located in Shahe Town, Changping District, Beijing, covering an area of over 70000 m^2. It was completed in March 2024. Six high-standard laboratory buildings have been newly built in the Shahe Technical Area, including DNA, criminal trace testing, forensic medicine, physical and chemical analysis and other specialties, reflecting the new concepts of building criminal technology laboratories.

Intelligent system design

The laboratory management system can intelligently manage the circulation and storage of evidence, the instruments and equipment, and the laboratory environment control. An intelligent data center has been built to provide unified supporting services for storing large amounts of technical data.

Infrastructure

Real time exhaust gas discharge and automatic replenishment of clean air have been achieved through the fresh air system. The laboratory has added uninterruptible power supply (UPS) and diesel generator backup power supply. A centralized gas supply system has been built to intelligently supply the required specialty gases.

Environment-friendly

The laboratory roofs are equipped with heat recovery devices to recover the cold and heat of exhaust gas, greatly reducing energy consumption. Reuse of the activated carbon adsorption devices and acid-base water washing towers classifies and treats the waste gas according to its properties. The technical area also has a reclaimed water station and a rainwater storage tank.

Humanistic care

The laboratory buildings are all designed in an independent Chinese courtyard style. The tea break zone, negotiation area, changing rooms, telephone booths, mother and baby rooms etc. have been set up in the public areas of each building to provide a comfortable and convenient working and living environment for technical staff.

The completion of Shahe Technical Area of IFS is an important step to accelerate the construction of international first-class forensic science institution for IFS, which is of great significance for comprehensively improving scientific research and innovation capabilities, practical support level, and talent cultivation quality.

Figure 1: Exterior of the Shahe Technical Area of IFS, China.

Figure 2: Inside of the Shahe Technical Area of IFS, China.

Evaluation of Optimal Techniques for the Recovery of Fingermark from the Adhesive Side of Black Electrical Tapes on Various Substrates

Ms Jodi Sim Siew Fen, Ms Jovi Low, Ms Li Shi Lim*
Forensics Division, Criminal Investigation Department, Singapore Police Force
Forensics CoE, Home Team Science and Technology, Singapore
**Email: Jodi_Sim@spf.gov.sg*

Abstract

Adhesive tapes play a significant role in criminal investigations because they effectively preserve fingerprint evidence. The adhesive side is particularly valuable for fingerprint recovery since it naturally fixes prints in place. When found at crime scenes, these tapes are often either stuck to themselves or adhered to other surfaces, requiring careful separation to examine the adhesive side for fingerprints. This separation needs to be handled carefully to avoid losing potential fingerprint evidence that could help identify perpetrators.

This study examined electrical tapes, which are frequently encountered as evidence in criminal cases, to determine the optimal methods for separating tape layers and developing fingermarks on the adhesive side. The work was conducted in two phases: Phase 1 investigated the optimal separation and fingermark development techniques on commonly encountered surfaces using porous (newspaper) and non-porous (non-adhesive side of tape) substrates to identify the most effective approach. Phase 2 then tested these successful methods on materials commonly found in the construction of Improvised Explosive Devices (IEDs), including bag interiors, metal poles and exposed wires. The results showed that using the pull and prise separation method, followed by treatment with adhesive-side developer, consistently yielded high-quality latent prints across all substrate types, except exposed wires.

Introduction

Tapes are commonly used in violent crimes to restrain the victims, drug packaging [1, 3], weapon concealment and for the construction of IEDs. During handling, the adhesive side of tapes often retains fingerprints of individuals connected to the incident [1, 4]. Successful recovery of these prints can be crucial in solving cases and strengthening prosecution [1]. Adhesive tapes are thus often analysed by forensic laboratories for the presence of physical evidence due to their association with a myriad of criminal activities [1-3] and security threats. Therefore, optimising fingermark development techniques is essential to maximise latent print recovery from the adhesive side of tapes.

As tapes often adhere to various surfaces, separating tapes from these surfaces is critical for fingermark development. Traditional methods including cooling, heating and chemical treatment have been used for the separation of tapes [1, 5]. Heptane, an organic solvent effective in reducing tape adhesion [6], is also commercially available as Un-Du® [7]. However, solvent-based methods can compromise print quality [5, 6]. Given that each tape separation method has its own limitations and drawbacks, there is a need to optimise and develop suitable separation methods for tapes adhered to various substrates.

Well-established fingermark development methods exist for the non-adhesive side of tape, but the adhesive side remains a challenge [3-4]. Commonly used powder methods are ineffective as the powder adheres to the entire sticky surface, resulting in poor contrast. However, several techniques like Gentian Violet, TapeGlo™ and Sticky-Side Powder™ can effectively develop fingermarks on adhesive surfaces, with their efficacy depending on the tape's type and colour [2, 4-5].

Processing dark-coloured tapes presents additional challenges for fingermark development due to poor contrast [2-4]. Methods like print transfer to photographic plates and superglue fuming with fluorescent dyes have

been explored, but these methods can be time-consuming and produce inconsistent results [2-4]. Current techniques for processing the adhesive side of dark-coloured electrical tapes have limitations in terms of providing adequate contrast between the fingermark and background, ease of application, shelf-life and the ability to develop fingermarks from such tapes adhered to different substrates [3-4]. Thus, improving fingermark development on dark adhesive tapes remains an active area of research.

This study aims to determine both the optimal tape separation and fingermark development method for the adhesive side of dark-coloured electrical tapes. This study also compares the efficiency of commonly used fingermark development methods to identify the most universal and effective method across different substrates.

Materials and Methods

Phase 1 *– Determining the optimal separation and fingermark development techniques for fingermarks on the adhesive side of electrical tape adhered to porous and non-porous substrates*

Tape Separation Techniques

This experiment evaluated three separation techniques: pull and prise (PP), Un-Du® (UN) and heptane (H). PP involves gradually pulling the tape away from the substrate, causing the adhesive layer to stretch until it detaches from the surface. In contrast, organic solvent methods (UN and H) work by applying the solvent between the adhesive layer and substrate, which softens the adhesive and reduces its adhesion to the surface.

Fingermark Development Techniques on the Adhesive Side of Electrical Tapes

Three distinct development techniques were evaluated in this study. The first technique employed TapeGlo™ (TG), a fluorescent dye that enables fingermark visualisation under an alternative light source (ALS). The second technique involved a two-step process: cyanoacrylate (CA) fuming followed by enhancement with basic yellow fluorescent dye, and subsequent visualisation under ALS. The third technique utilised adhesive side developer (AD), a powder suspension. Given the use of dark-coloured electrical tapes in this experiment, a light-coloured AD was selected to maximise contrast, allowing direct visual examination without additional light sources.

Experimental Set-Up

The effectiveness of the tape separation and fingermark development techniques was evaluated using two types of substrates: porous (newspaper) and non-porous (non-adhesive side of tape). Nine combinations of separation and development techniques were tested – PPTG, PPCA, PPAD, UNTG, UNCA, UNAD, HTG, HCA, and HAD.

For each substrate, five donors were asked to deposit one thumbprint on each of the nine pieces of pre-cut dark-coloured electrical tapes. The donors first washed and dried their hands, then coated their thumb with a layer of facial oil by rubbing it against their forehead and/or nose. They deposited a thumbprint in the middle of each tape before lifting their thumb in a single upward motion. This process was repeated for all nine tapes per donor.

Each tape was then cut lengthwise, with the left half (labelled “A”) serving as reference sample and right half (labelled “B”) serving as test sample. The reference samples were stored in an enclosed space, while the test samples were adhered to the substrate. After three days, the reference samples underwent direct fingermark development technique, while the test samples were first separated from the substrate they were adhered to using the designated separation technique, before fingermark development.

Table 1: Labelling of reference and test samples for each donor

Method	Donor 1	
PPTG	1A	1B
PPCA	2A	2B
PPAD	3A	3B
UNTG	4A	4B
UNCA	5A	5B
UNAD	6A	6B
HTG	7A	7B
HCA	8A	8B
HAD	9A	9B

Method	Donor 2	
PPTG	11A	11B
PPCA	12A	12B
PPAD	13A	13B
UNTG	14A	14B
UNCA	15A	15B
UNAD	16A	16B
HTG	17A	17B
HCA	18A	18B
HAD	19A	19B

Method	Donor 3	
PPTG	21A	21B
PPCA	22A	22B
PPAD	23A	23B
UNTG	24A	24B
UNCA	25A	25B
UNAD	26A	26B
HTG	27A	27B
HCA	28A	28B
HAD	29A	29B

Method	Donor 4	
PPTG	31A	31B
PPCA	32A	32B
PPAD	33A	33B
UNTG	34A	34B
UNCA	35A	35B
UNAD	36A	36B
HTG	37A	37B
HCA	38A	38B
HAD	39A	39B

Method	Donor 5	
PPTG	41A	41B
PPCA	42A	42B
PPAD	43A	43B
UNTG	44A	44B
UNCA	45A	45B
UNAD	46A	46B
HTG	47A	47B
HCA	48A	48B
HAD	49A	49B

Scoring of Fingermarks

Each reference and test sample pair were photographed under appropriate conditions (using ALS for TapeGlo™ or fluorescent-dyed cyanoacrylate tapes). The photographs were then forwarded to five fingerprint examiners for independent assessment. The examiners were allowed to digitally enhance the images and scored the fingermarks using the international "CAST" (Centre for Applied Science & Technology) scale.

Table 2: International "CAST" scale

Grade	Level of Detail
0	No evidence of a fingermark
1	Some evidence of a fingermark
2	Less than 1/3 clear ridge detail
3	Between 1/3 and 2/3 clear ridge detail
4	Over 2/3 clear ridge detail

Phase 2 *– Testing optimal techniques identified from Phase 1 on substrates commonly found in IED construction*

Optimal Techniques Established from Phase 1

Based on Phase 1 results, the pull and prise separation method followed by adhesive side developer (PPAD) proved most effective and was selected for Phase 2 testing. This phase examined substrates commonly found in IED construction, which were identified by our in-house Bomb Technical Investigation Team (BTIT), as bag interiors, metal poles and exposed wires.

Experimental Set-Up

Phase 2 incorporated realistic handling conditions through donor simulation. The same donors from Phase 1 were each provided with a roll of dark-coloured electrical tape and instructed to cut nine pieces (5cm each), before adhering three pieces to each substrate (i.e. bag interiors, metal poles and exposed wires). Donors were allowed to handle the test samples naturally without restrictions. A BTIT officer cut three reference samples (one for each substrate) whilst simulating typical bomb-making handling patterns. All tapes were subsequently processed using PPAD and photographed individually.

As the donor-simulated tapes contained multiple fingermarks, each identifiable fingermark was marked (Figure 1) to ensure that all examiners evaluated the same prints using the CAST scale.

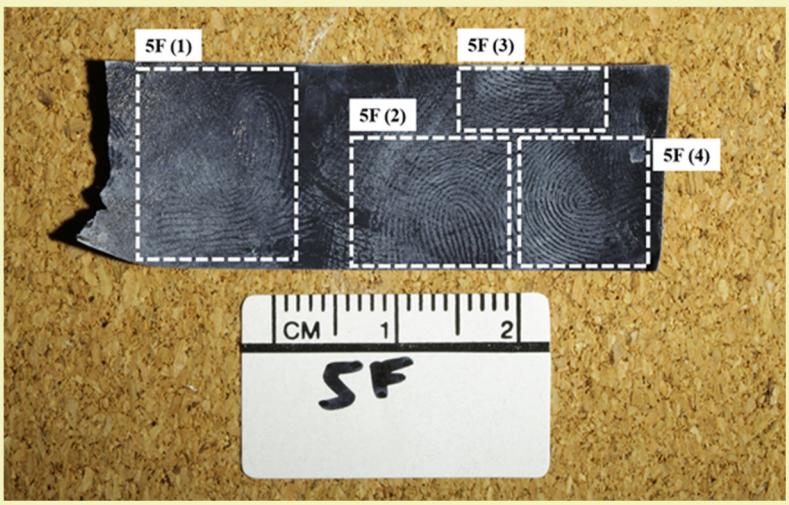


Figure 1: Identifiable fingermarks marked out on a donor-simulated tape for standardisation of scoring

Results and Discussion

Table 3 shows the mean fingermark quality scores across all donors, as assessed by different examiners during Phase 1. Comparing these results, TG consistently produced lower quality scores, whereas both CA and AD methods achieved minimum scores of 3.0. These findings demonstrate that TG exhibited reduced effectiveness for latent print development even prior to tape separation, in contrast to the higher quality results obtained through the CA and AD methodologies. Images of the developed prints are appended in Appendix A.

Table 3: A summary of mean fingermark quality scores from reference and test samples.

Substrate	Fingermark Development Method	Mean Score for Reference Samples (A)	Separation Method (1)	Fingermark Development Method (2)	Mean Score for Test Samples (B) [B=1+2]
Non-adhesive Side of Tape (Non-porous)	TG	1.76	PP	TG	1.56
		1.76	UN	TG	1
		1.84	H	TG	1
	CA	3.2	**PP**	**CA**	**2.76**
		2.8	UN	CA	1
		3.16	H	CA	1
	AD	3.68	**PP**	**AD**	**2.12**
		3.8	UN	AD	1
		3.64	H	AD	1
Newspaper (Porous)	TG	2.08	PP	TG	1.44
		2.04	UN	TG	1
		1.32	H	TG	1
	CA	3.68	**PP**	**CA**	**2.2**
		3.4	UN	CA	1
		3.2	H	CA	1
	AD	3.64	**PP**	**AD**	**2.8**
		3.68	UN	AD	1.16
		3.68	H	AD	1.04

All experimental tapes requiring substrate separation demonstrated decreased fingermark quality as compared to reference tapes that were unattached to substrates. The UN and H separation methods yielded predominantly 1.0 ratings, with an average quality reduction of approximately 50%, despite the initial fingermark deposits exhibiting between 1/3 and 2/3 clear ridge detail (Figure 2).

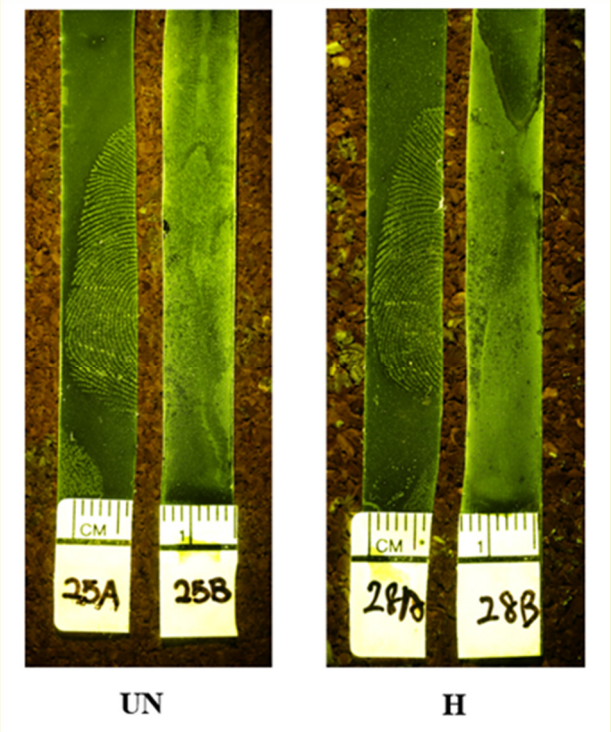


Figure 2: Solvent-based separation methods resulted in significant degradation of fingermark quality on the test samples (tapes labelled "B") compared with corresponding reference samples (tapes labelled "A")

Non-porous substrate (Non-adhesive Side of Tape):

Of the tested methods, PPCA achieved the highest score (2.76) for fingermarks developed from tapes pasted on the non-adhesive side of tape. However, caution must be exercised when applying CA fuming methods to tapes that have had contact with water, as this could potentially obscure fingermarks. PPAD demonstrated broader applicability than PPCA, particularly in processing wetted tapes and offered greater ease of application.

Porous substrate (Newspaper):

For tapes adhered to newspaper, PP demonstrated better performance than solvent-based methods, despite potential newspaper residue adhesion, followed by AD application (Figure 3). In this context, PP should be performed with care to prevent over-etching of the newspaper.

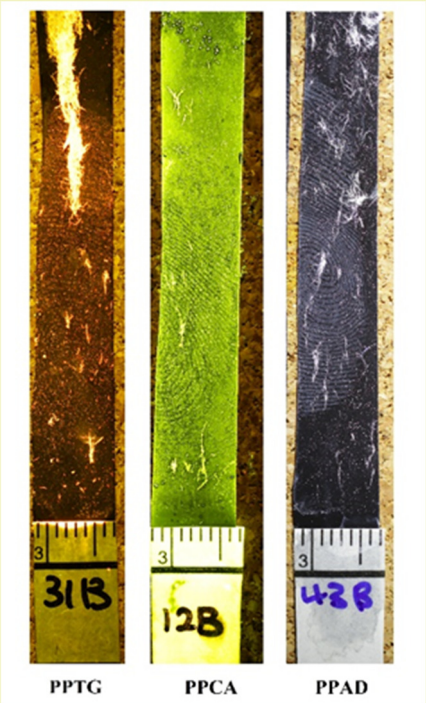


Figure 3: Test samples after PP separation from newspaper followed by different fingermark development methods

Phase 1 results established PPCA and PPAD as the optimal methods for separation and fingermark development on non-porous and porous substrates, respectively. As the mean scores for these combinations are relatively close and fall between Grades 2 and 3 on the 'CAST' scale (Table 2), PPAD proves superior to PPCA overall, as it eliminates the need to determine whether the tape has been previously exposed to moisture before processing.

Table 4 presents the data obtained from Phase 2. The mean scores across donors demonstrated that PPAD successfully develops prints of at least Grade 2 quality for tapes adhered to the interior of bag or metal pole, but not on exposed wires (prints were mostly Grade 0). Images of the developed prints are appended in Appendix B.

Table 4: A summary of latent fingermark results from test and reference samples on substrates commonly encountered in the construction of IEDs, processed using PPAD.

Separation and Fingermark Development Method	Substrate	Test Samples (mean score across all donors)	Reference Samples (prepared by BTIT)
PPAD	Interior of Bag	2.28	3.4
	Metal Pole	2.42	3.8
	Exposed Wires	0.23	0

Based on BTIT's simulations of how tapes are typically handled during IED construction -drawing on their extensive expertise in this area - the reference samples they prepared were expected to achieve higher scores than the test samples. This expectation stemmed from BTIT personnel's familiarity with handling such components, resulting in a lower likelihood of overlapping fingermarks. Nevertheless, the results remained consistent, demonstrating that PPAD performed most effectively on metal poles, followed by bag interiors, while proving ineffective on exposed wires (maintaining Grade 0).

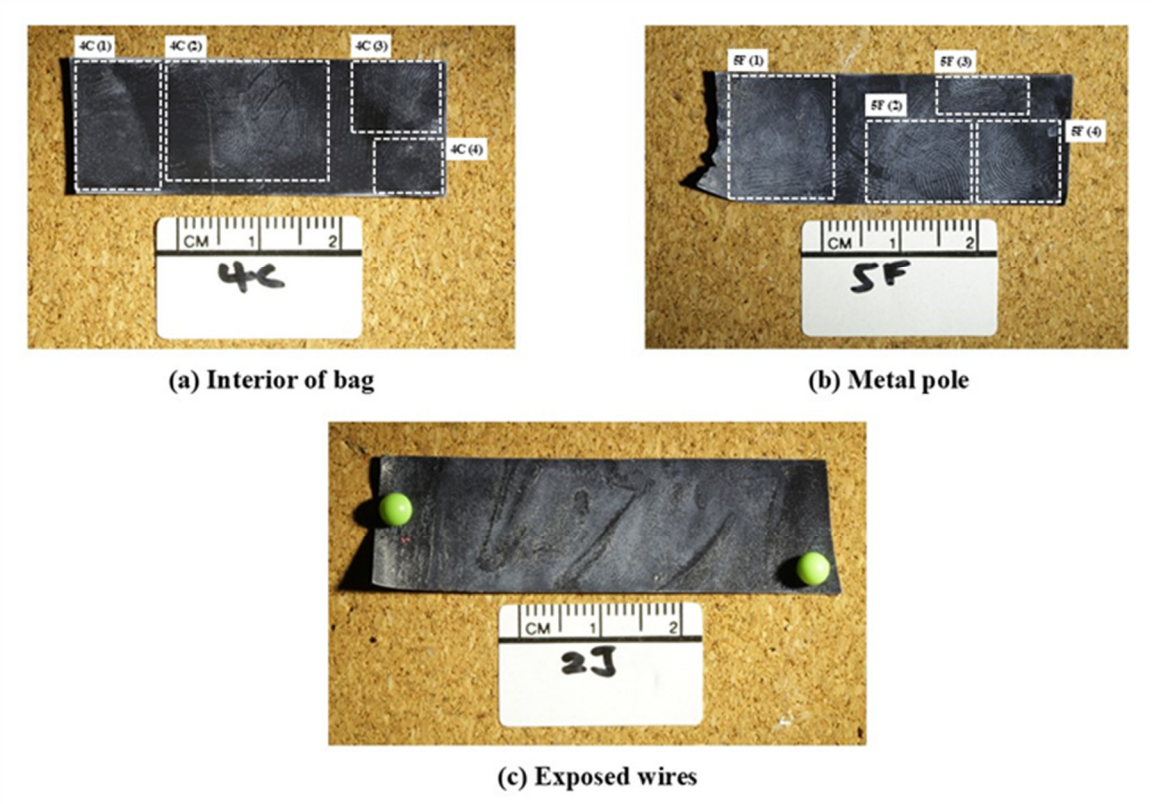


Figure 4: Tapes with identifiable fingermarks marked out (where present), after separation and development with AD: (a) interior of bag, (b) metal pole and (c) exposed wires

Compared to other substrates, exposed wires presented an extremely small surface area and the inevitable self-adherence of the tape impeded the separation process, resulting in minimal print recovery. This is evident in Figure 4(c), which shows visible background disturbance on the adhesive surface caused by physical wounding of the tape around exposed wires, in contrast to tapes adhered to bag interiors and metal poles, which offered larger and flatter surfaces.

Setting aside external factors such as tape handling methods or substrate characteristics that could compromise fingermark quality before separation and development, PPAD has consistently produced quality prints on both non-porous and porous substrates, as well as on materials commonly encountered in IED construction, with the exception of exposed wires. This observation is based on all the data gathered from the study.

Conclusion and Future Work

Various separation and development methods were explored on the adhesive side of dark-coloured electrical tapes adhered to different substrates (newspaper, non-adhesive side of tapes, bag interiors, metal poles and exposed wires). PPAD consistently yielded high-quality prints across all tested substrates, except for exposed wires. The results observed in this study are in agreement with earlier findings regarding UN and H; while effective for tape removal, these solvent-based methods resulted in notable deterioration of print quality. Thus, preliminary testing on similar materials before processing actual evidence is crucial. This approach allows for method optimisation and helps prevent potential evidence loss.

Moving forward, while this study primarily focused on electrical tapes, future research could encompass other varieties such as duct tape and masking tape. Additionally, further testing is necessary to examine electrical tape remnants from improvised explosive device (IED) construction in post-blast scenarios. This expansion is essential, as the parameters established for electrical tapes may not apply universally due to different chemical and physical properties of tape adhesives. As this study involved relatively fresh three-day-old prints, a time-dependent experiment could be further conducted using our recommended parameters to assess the results for tapes and

prints that have been adhered or deposited for longer periods of time. This would help validate our results across a broader range of print ages. These future directions would provide a more comprehensive understanding of current tape processing methodologies and contribute to maximising evidence recovery from various tape types under different conditions, ultimately advancing the scientific rigour of forensic investigations.

Acknowledgements

The authors gratefully acknowledge the assistance of officers from the Fingerprint Examination Team (FET) and Bomb Technical Investigation Team (BTIT) for their valuable contributions to this study. We thank Ms Lee Mei Fun, Mr Mohd Yazid, Mr Yong Heng Wei, Ms Chen Yew Poh and Mr Eng Cher Lip from FET for their expertise in fingermark quality assessment, and Mr. Sito Wai Yip from BTIT for providing technical guidance on IED tape components.

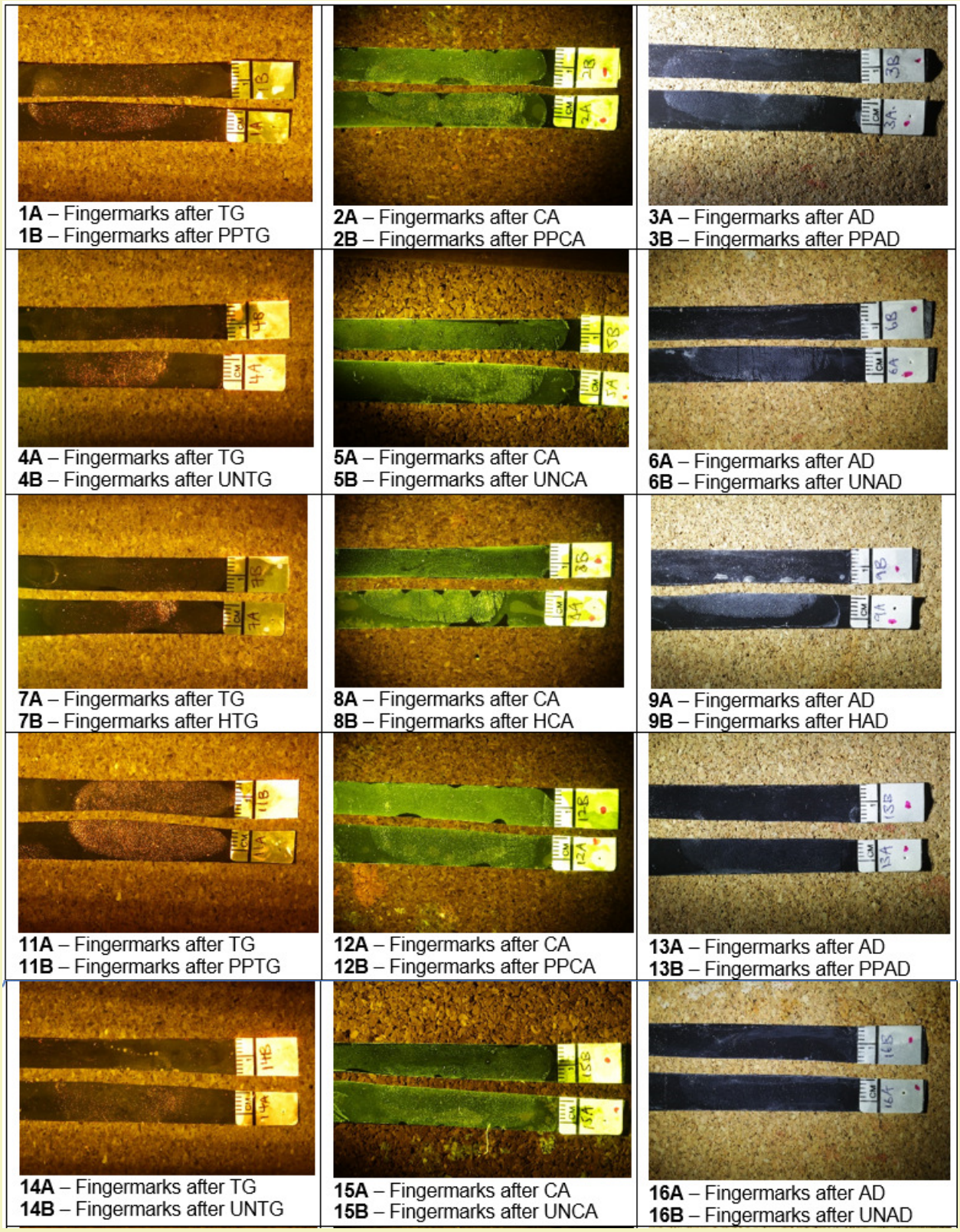
References:

1) *Kim S, Hong S. A comparison of adhesive tape-separation methods from surfaces; dipping in liquid nitrogen, liquid nitrogen spray and an adhesive neutralizer method. J Forensic Res. 2016;7(5).*
2) *Maldonado A, Rushton C, Staton P, et al. The simultaneous development of latent prints on the adhesive and non-adhesive sides of tape using a Rhodamine 6G in Tween 20 solution after cyanoacrylate fuming [oral presentation]. American Academy of Forensic Sciences; 2013; Seattle, WA. Marshall University Forensic Science Center, Seminar Project.*
3) *Brzozowski J, Białek I, Subik P. Visualisation of fingerprints on sticky side of adhesive tapes. Probl Forensic Sci. 2005;Pa:333-42.*
4) *Jasuja O, Singh G, Sodhi G. Development of latent fingerprints on the sticky side of adhesive tapes: phase transfer catalyst-based formulation. Can Soc Forensic Sci J. 2007;40(1):1-13. doi:10.1080/00085030.2007.10757147.*
5) *Bailey J, Crane J. Use of nitrogen cryogun for separating duct tape and recovery of latent fingerprints with a powder suspension method. Forensic Sci Int. 2011;210(1-3):170-3. doi:10.1016/j.forsciint.2011.03.004.*
6) *Molina D. The use of Un-Du® to separate adhesive materials. J Forensic Ident. 2007;57:688-96.*
7) *About Un-Du® [Internet]. Un-du.com; 2019 [cited 2019 Sep 27]. Available from: https://www.un-du.com/About-Us.html.*

Appendix A:

Phase 1 Results – Fingermarks developed following separation and/or fingermarks development techniques

Tapes pasted on Non-adhesive Side of Tape (Non-porous)



1A – Fingermarks after TG
1B – Fingermarks after PPTG

2A – Fingermarks after CA
2B – Fingermarks after PPCA

3A – Fingermarks after AD
3B – Fingermarks after PPAD

4A – Fingermarks after TG
4B – Fingermarks after UNTG

5A – Fingermarks after CA
5B – Fingermarks after UNCA

6A – Fingermarks after AD
6B – Fingermarks after UNAD

7A – Fingermarks after TG
7B – Fingermarks after HTG

8A – Fingermarks after CA
8B – Fingermarks after HCA

9A – Fingermarks after AD
9B – Fingermarks after HAD

11A – Fingermarks after TG
11B – Fingermarks after PPTG

12A – Fingermarks after CA
12B – Fingermarks after PPCA

13A – Fingermarks after AD
13B – Fingermarks after PPAD

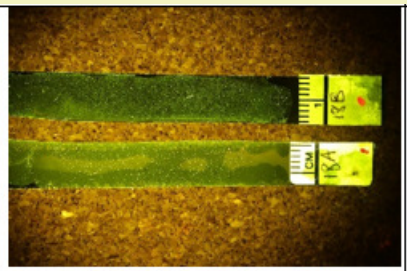
14A – Fingermarks after TG
14B – Fingermarks after UNTG

15A – Fingermarks after CA
15B – Fingermarks after UNCA

16A – Fingermarks after AD
16B – Fingermarks after UNAD

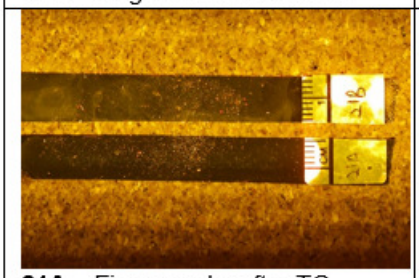
Appendix A (Cont'd):

17A – Fingermarks after TG
17B – Fingermarks after HTG

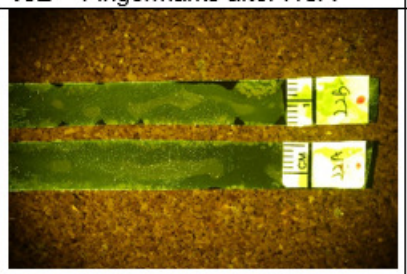


18A – Fingermarks after CA
18B – Fingermarks after HCA

19A – Fingermarks after AD
19B – Fingermarks after HAD

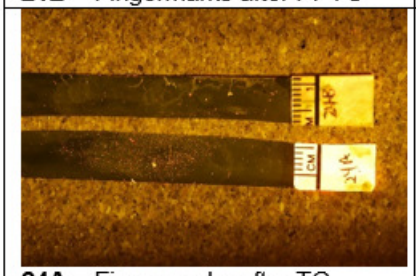


21A – Fingermarks after TG
21B – Fingermarks after PPTG

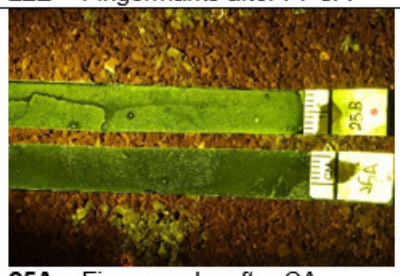


22A – Fingermarks after CA
22B – Fingermarks after PPCA

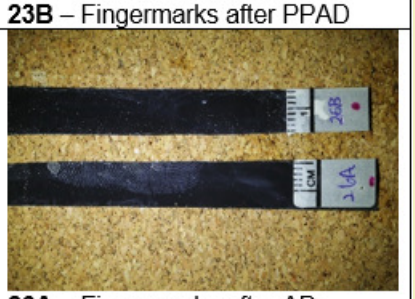
23A – Fingermarks after AD
23B – Fingermarks after PPAD



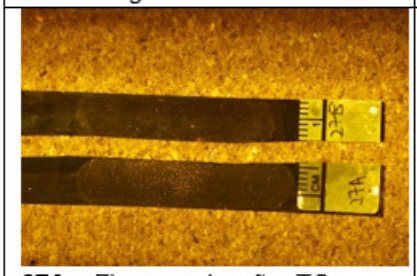
24A – Fingermarks after TG
24B – Fingermarks after UNTG



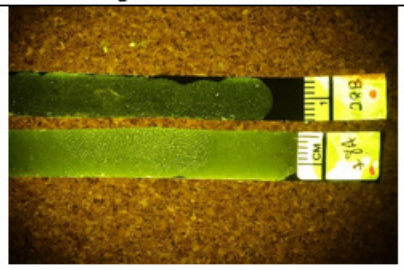
25A – Fingermarks after CA
25B – Fingermarks after UNCA



26A – Fingermarks after AD
26B – Fingermarks after UNAD

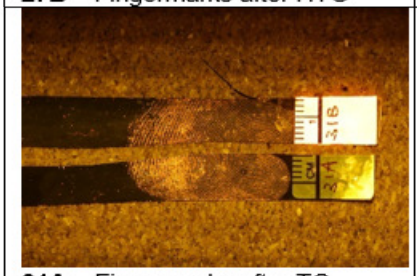


27A – Fingermarks after TG
27B – Fingermarks after HTG



28A – Fingermarks after CA
28B – Fingermarks after HCA

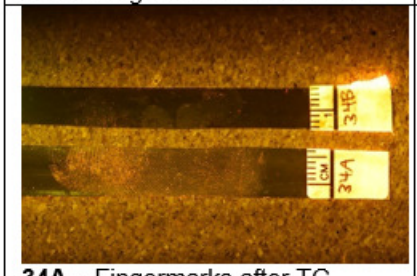
29A – Fingermarks after AD
29B – Fingermarks after HAD



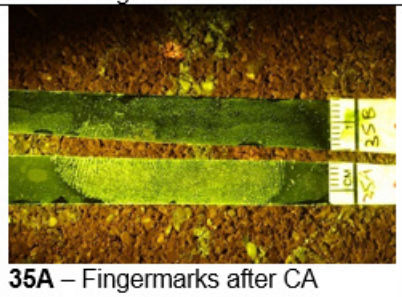
31A – Fingermarks after TG
31B – Fingermarks after PPTG

32A – Fingermarks after CA,
32B – Fingermarks after PPCA

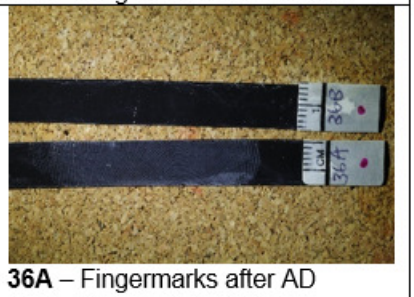
33A – Fingermarks after AD
33B – Fingermarks after PPAD



34A – Fingermarks after TG
34B – Fingermarks after UNTG

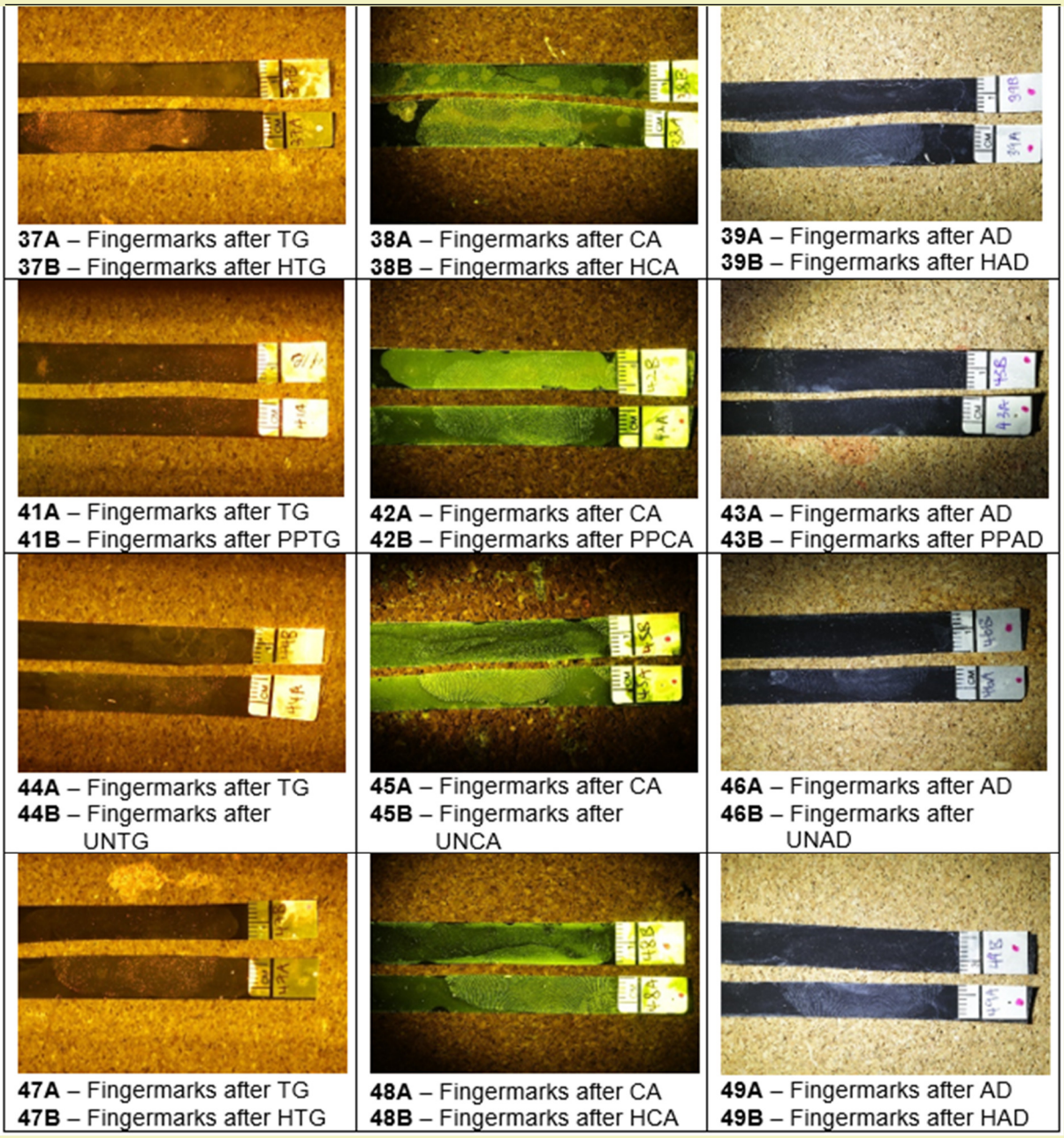


35A – Fingermarks after CA
35B – Fingermarks after UNCA



36A – Fingermarks after AD
36B – Fingermarks after UNAD

Appendix A (Cont'd):



37A – Fingermarks after TG
37B – Fingermarks after HTG

38A – Fingermarks after CA
38B – Fingermarks after HCA

39A – Fingermarks after AD
39B – Fingermarks after HAD

41A – Fingermarks after TG
41B – Fingermarks after PPTG

42A – Fingermarks after CA
42B – Fingermarks after PPCA

43A – Fingermarks after AD
43B – Fingermarks after PPAD

44A – Fingermarks after TG
44B – Fingermarks after UNTG

45A – Fingermarks after CA
45B – Fingermarks after UNCA

46A – Fingermarks after AD
46B – Fingermarks after UNAD

47A – Fingermarks after TG
47B – Fingermarks after HTG

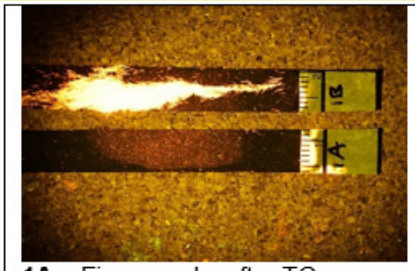
48A – Fingermarks after CA
48B – Fingermarks after HCA

49A – Fingermarks after AD
49B – Fingermarks after HAD

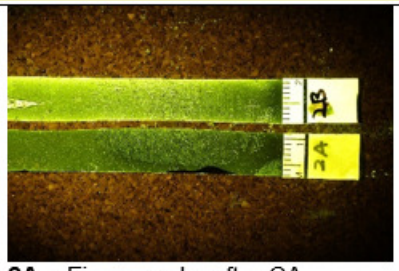
Appendix A (Cont'd):

Phase 1 Results – Fingermarks developed following separation and/or fingermarks development techniques

Tapes pasted on Newspaper (Porous)

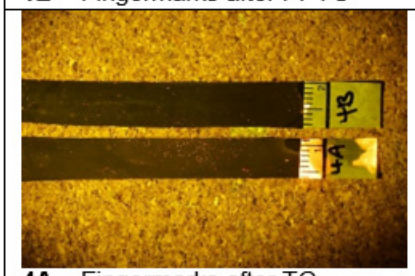


1A – Fingermarks after TG
1B – Fingermarks after PPTG



2A – Fingermarks after CA
2B – Fingermarks after PPCA

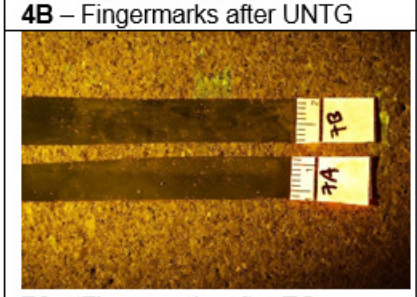
3A – Fingermarks after AD
3B – Fingermarks after PPAD



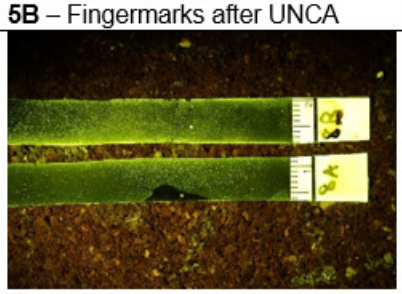
4A – Fingermarks after TG
4B – Fingermarks after UNTG

5A – Fingermarks after CA
5B – Fingermarks after UNCA

6A – Fingermarks after AD
6B – Fingermarks after UNAD

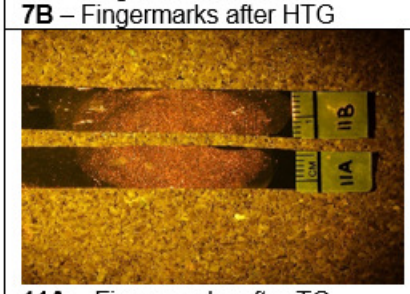


7A – Fingermarks after TG
7B – Fingermarks after HTG

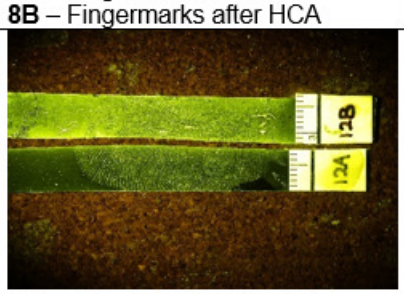


8A – Fingermarks after CA
8B – Fingermarks after HCA

9A – Fingermarks after AD
9B – Fingermarks after HAD

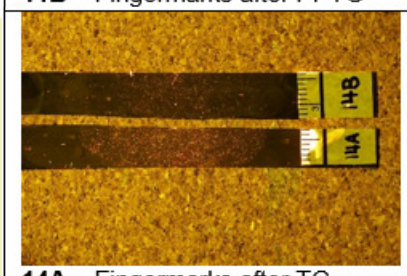


11A – Fingermarks after TG
11B – Fingermarks after PPTG

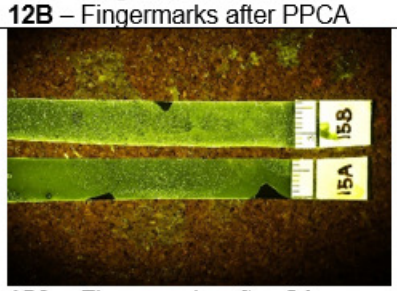


12A – Fingermarks after CA
12B – Fingermarks after PPCA

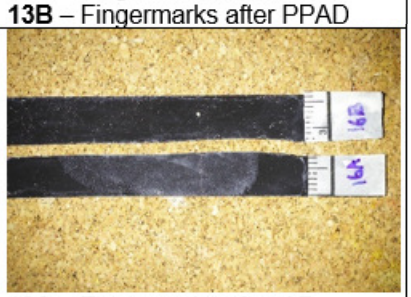
13A – Fingermarks after AD
13B – Fingermarks after PPAD



14A – Fingermarks after TG
14B – Fingermarks after UNTG

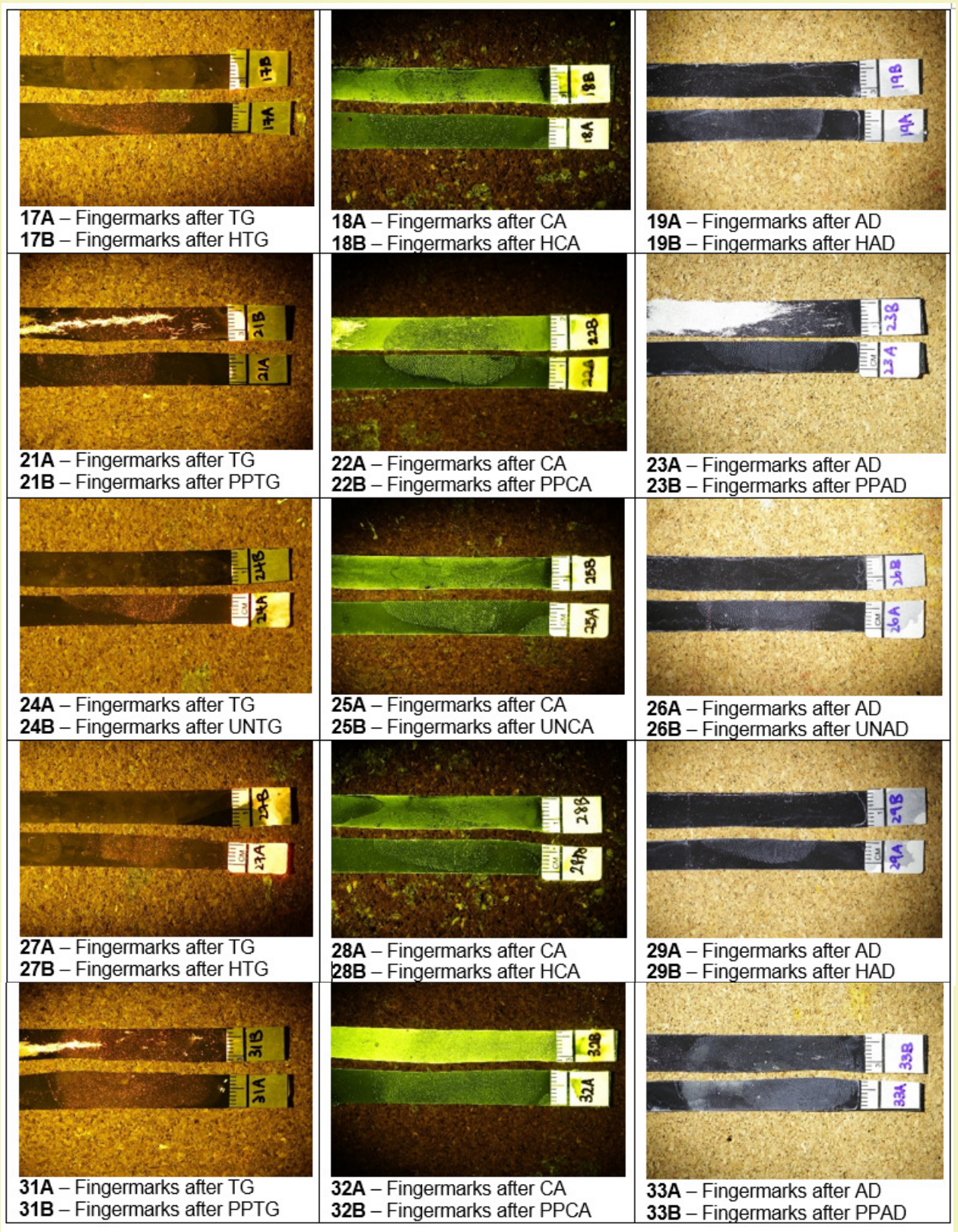


15A – Fingermarks after CA
15B – Fingermarks after UNCA



16A – Fingermarks after AD
16B – Fingermarks after UNAD

Appendix A (Cont'd):



17A – Fingermarks after TG
17B – Fingermarks after HTG

18A – Fingermarks after CA
18B – Fingermarks after HCA

19A – Fingermarks after AD
19B – Fingermarks after HAD

21A – Fingermarks after TG
21B – Fingermarks after PPTG

22A – Fingermarks after CA
22B – Fingermarks after PPCA

23A – Fingermarks after AD
23B – Fingermarks after PPAD

24A – Fingermarks after TG
24B – Fingermarks after UNTG

25A – Fingermarks after CA
25B – Fingermarks after UNCA

26A – Fingermarks after AD
26B – Fingermarks after UNAD

27A – Fingermarks after TG
27B – Fingermarks after HTG

28A – Fingermarks after CA
28B – Fingermarks after HCA

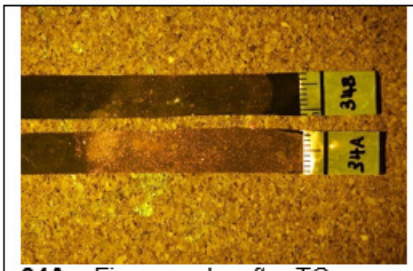
29A – Fingermarks after AD
29B – Fingermarks after HAD

31A – Fingermarks after TG
31B – Fingermarks after PPTG

32A – Fingermarks after CA
32B – Fingermarks after PPCA

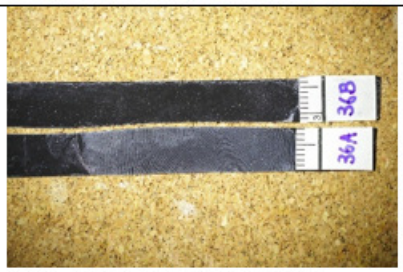
33A – Fingermarks after AD
33B – Fingermarks after PPAD

Appendix A (Cont'd):

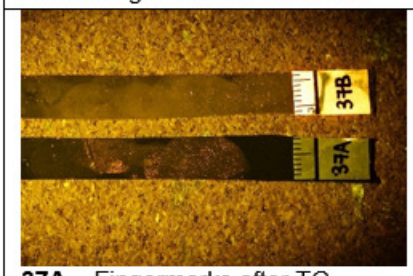


34A – Fingermarks after TG
34B – Fingermarks after UNTG

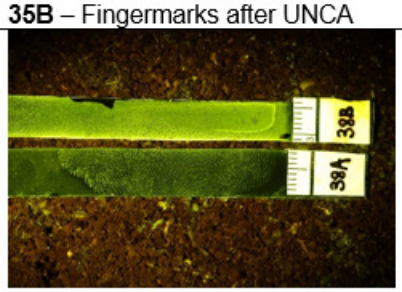
35A – Fingermarks after CA
35B – Fingermarks after UNCA



36A – Fingermarks after AD
36B – Fingermarks after UNAD

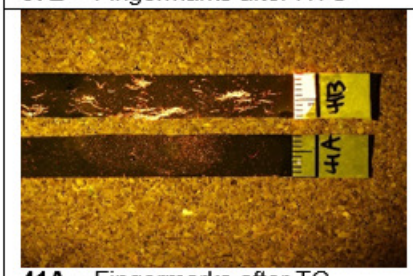


37A – Fingermarks after TG
37B – Fingermarks after HTG

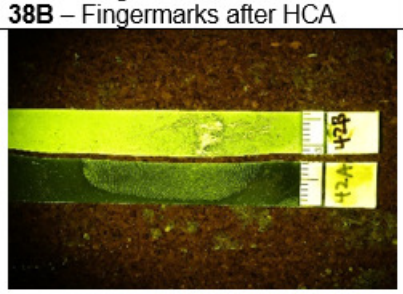


38A – Fingermarks after CA
38B – Fingermarks after HCA

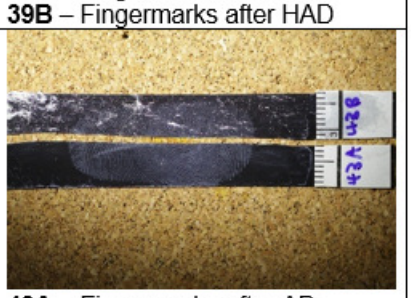
39A – Fingermarks after AD
39B – Fingermarks after HAD



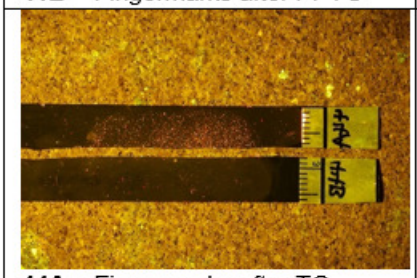
41A – Fingermarks after TG
41B – Fingermarks after PPTG



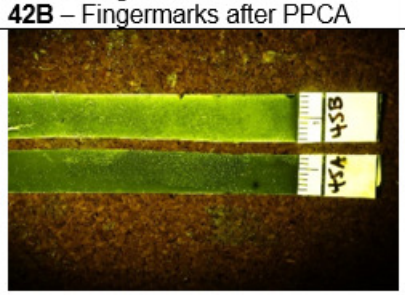
42A – Fingermarks after CA
42B – Fingermarks after PPCA



43A – Fingermarks after AD
43B – Fingermarks after PPAD

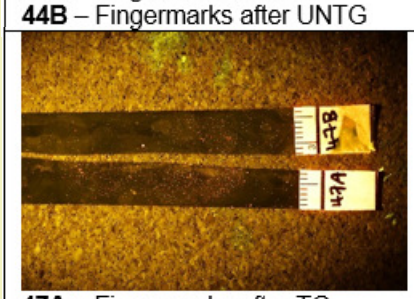


44A – Fingermarks after TG
44B – Fingermarks after UNTG

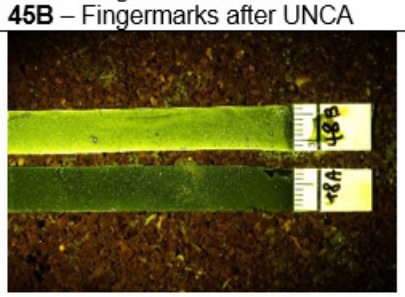


45A – Fingermarks after CA
45B – Fingermarks after UNCA

46A – Fingermarks after AD
46B – Fingermarks after UNAD



47A – Fingermarks after TG
47B – Fingermarks after HTG



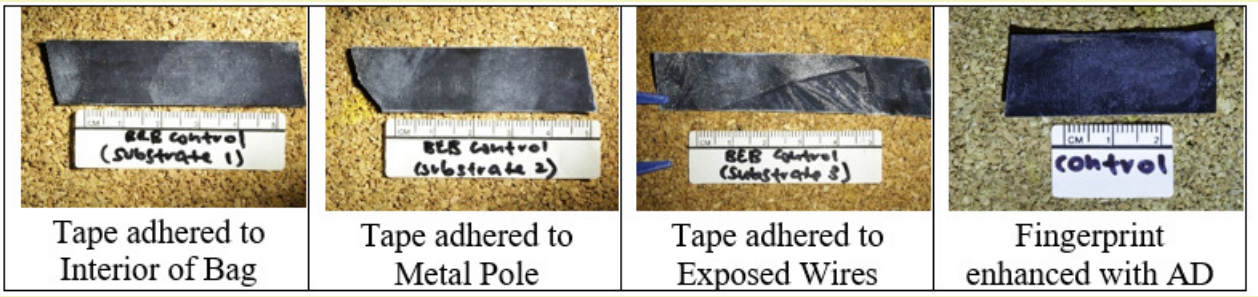
48A – Fingermarks after CA
48B – Fingermarks after HCA

49A – Fingermarks after AD
49B – Fingermarks after HAD

Appendix B:

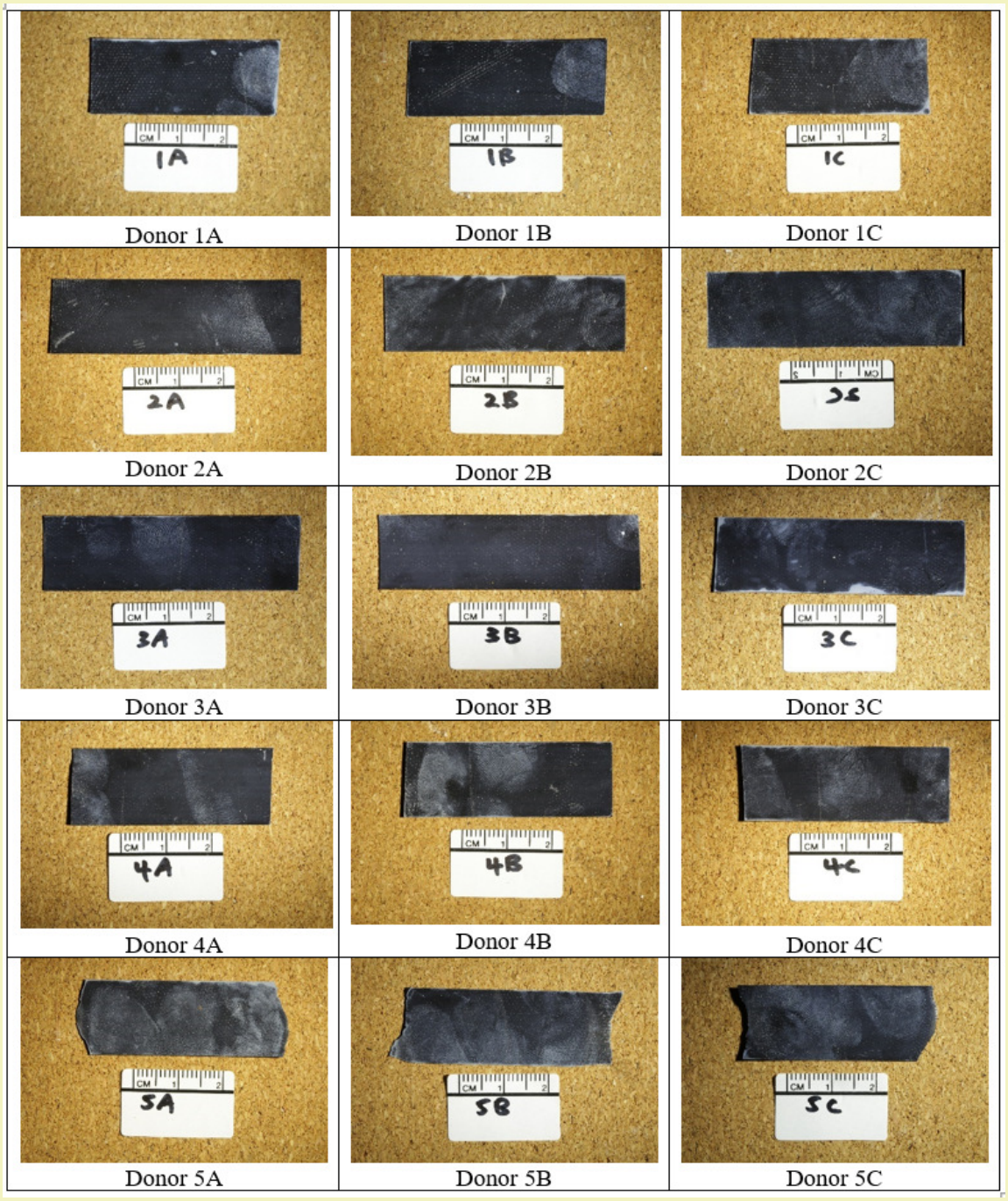
Phase 2 Results – Fingermarks developed from natural donor handling

Tapes handled by in-house BTIT



Tape adhered to Interior of Bag | Tape adhered to Metal Pole | Tape adhered to Exposed Wires | Fingerprint enhanced with AD

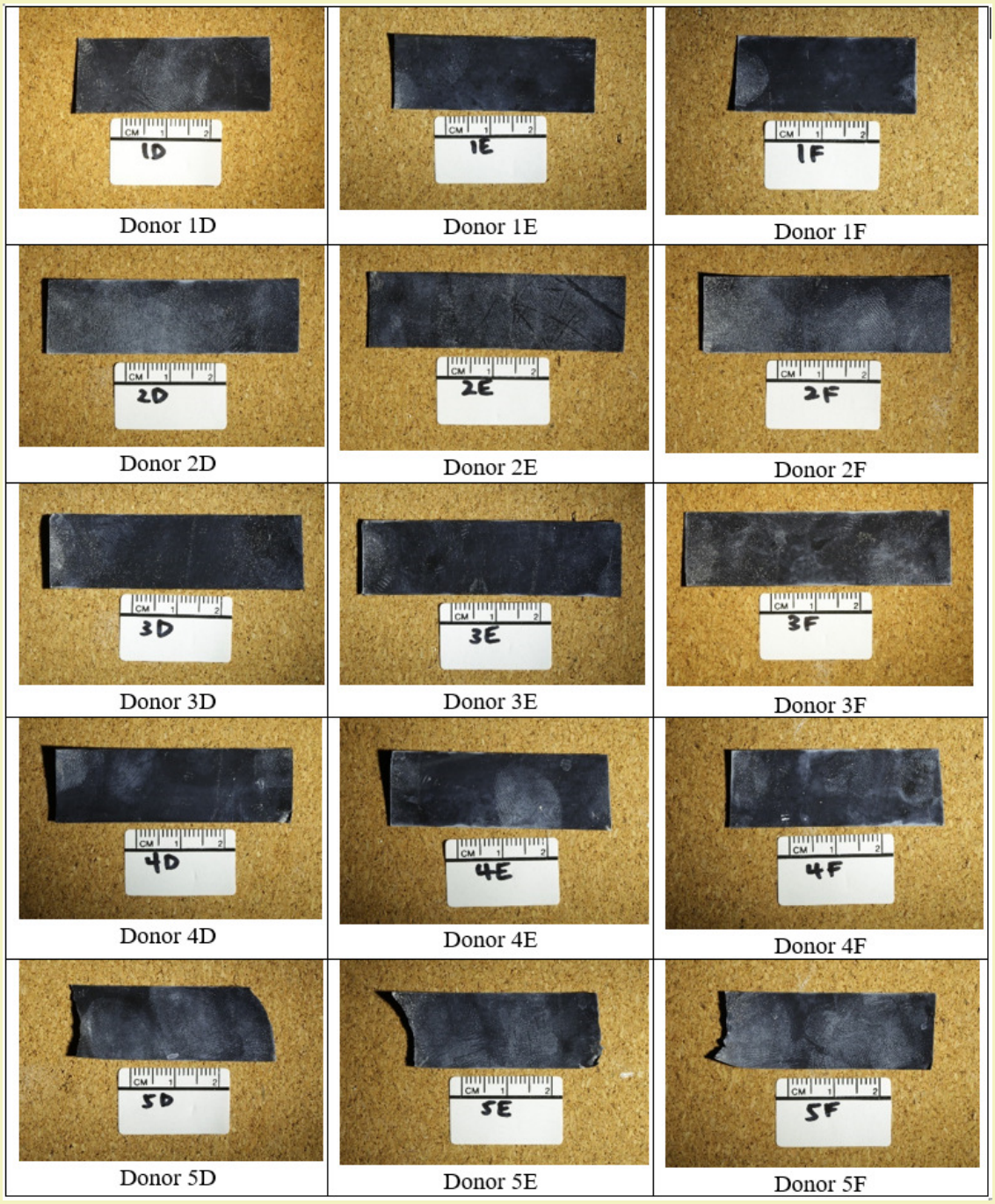
Tapes adhered to Interior of Bag



Donor 1A | Donor 1B | Donor 1C
Donor 2A | Donor 2B | Donor 2C
Donor 3A | Donor 3B | Donor 3C
Donor 4A | Donor 4B | Donor 4C
Donor 5A | Donor 5B | Donor 5C

Appendix B (Cont'd):

Tapes adhered to Metal Pole



Donor 1D | Donor 1E | Donor 1F

Donor 2D | Donor 2E | Donor 2F

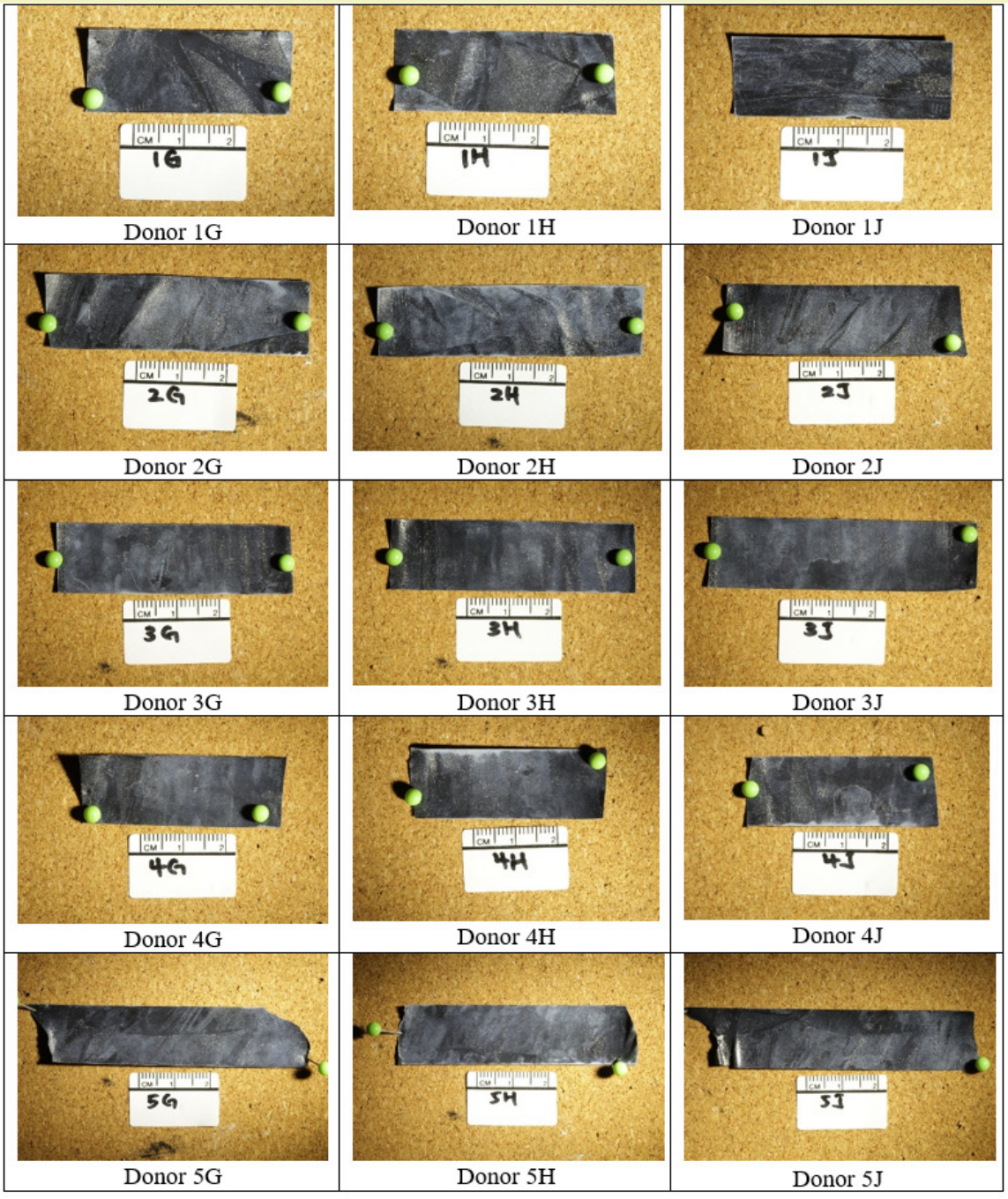
Donor 3D | Donor 3E | Donor 3F

Donor 4D | Donor 4E | Donor 4F

Donor 5D | Donor 5E | Donor 5F

Appendix B (Cont'd):

Tapes adhered to Exposed Wires



Donor 1G | Donor 1H | Donor 1J
Donor 2G | Donor 2H | Donor 2J
Donor 3G | Donor 3H | Donor 3J
Donor 4G | Donor 4H | Donor 4J
Donor 5G | Donor 5H | Donor 5J

'Unveiling the Invisible' - A Preliminary Study on Detecting Gunshot Residues on Dark Fabrics Using the Video Spectral Comparator

Mrs M Eranthi Jalika Seneviratne, Mr. W M Ajith Kumara*
Government Analyst's Department, Sri Lanka
**Email: eranthijs@gmail.com*

Abstract

Gunshot Residues (GSR) are expelled from the openings of a firearm during a discharge. Identification of GSR plays an important role in firearms related crimes. Due to the lack of contrast, visualization of GSR is difficult on dark fabrics. Partially burnt and unburnt gunpowder possess luminescent properties, this property can be used to visualize GSR deposited on dark fabrics using the Video Spectral Comparator (VSC) technique.

In this study, the propellants of two types of cartridges were examined to analyse their luminescent properties. The distribution patterns of GSR were evaluated based on two key factors: the type of firearm used and the distance between the firearm and the target.

The resulting images obtained from the VSC confirmed that the GSR distribution depends on the type of firearm and can potentially be used to differentiate the type of the firearm. Furthermore, the results confirmed that the GSR distribution varies with the distance. GSR distribution could be significantly identified up to the distance of 80 cm for the revolver used and up to 40 cm for the pistol used.

Introduction

Gunshot Residues and It's Importance

During a discharge of a firearm, primer residues, gunpowder residues and metal particles from projectiles and the cartridge case are expelled from the muzzle end and from other openings of the firearm. These residues are referred as Gunshot Residues (GSR) [1]. GSR provides an important evidentiary value in firearm related crimes [2].

The distribution of GSR around the bullet hole depends on many factors such as the firearm, the ammunition, the particle size and the shape of the propellant and on the shooting distance [3]. The GSR pattern also depends on factors such as the length of the barrel, firing angle, target composition and the atmospheric conditions [4].

Gunpowder particles exiting the muzzle end of a firearm, disperse in a conical shape as it moves further and further [5]. Generally, larger particles will travel a longer distance than smaller particles through air resistance. As shooting distance increases, only heaviest particles of burnt and unburnt gunpowder reach the target [6].

Some GSR particles can be visible by the naked eye or with a slight magnification if the background provides the necessary contrast. GSR may appear as translucent off-white-coloured particles, or they may appear as opaque dark-grey-coloured particles if they are coated with graphite. If the background does not provide necessary contrast, GSR may not visible by the naked eye. Colour developing chemical testing or Infra-Red photography are required to detect GSR in such cases [3], [5].

Infra-Red luminescent characteristics of propellants to identify GSR

The majority of GSR consists of burnt and unburnt particles from the propellant and the primer. Several earlier studies have shown that they have luminescent properties towards the IR spectrum [3], [7], [8]. Therefore, alternative light sources (ALS) have been used by many scientists to identify GSR. The VSC is an imaging device which is commonly used in forensic questioned documents examination laboratories to examine altered and counterfeit documents. In 2006, Atwater et al. suggests visualizing the GSR patterns on dark clothing quickly and easily using the VSC under IR radiation [7].

Objectives of the Study

As previously mentioned, visualization of GSR on dark targets is challenging due to the lack of contrast. Although well-established methods such as the Modified-Griess test can be applied to identify GSR on dark garments, a non-destructive method to identify the presence of GSR would be an advantage.

This study was designed to evaluate the possibility of using the VSC to visualize GSR distribution on dark fabrics. Additionally, the study aimed to evaluate the influence of two primary factors—firearm type and shooting distance—on GSR distribution.

The effect of the firearm on the GSR distribution pattern was studied by carrying out test trials with four different handguns at the distance of 10 cm. The effect of the distance on GSR distribution pattern was studied by carrying out test trials with two handguns at five distances (10 cm, 20 cm, 40 cm, 80 cm and 120 cm).

Methodology

The methodology used involves several steps to ensure controlled and reliable data collection and analysis.

Firearms Examination

Initially, four types of firearms used for the study were examined for their characteristics
1) Arminius Long barrel revolver (.38 Special)
2) Arminius short barrel revolver (.38 Special)
3) CZ 85 B Pistol (9×19mm)
4) Norinco NP 22 Pistol (9×19mm)

Propellant Analysis

One cartridge each from two types of cartridges used (.38 special, 9×19mm) were disassembled and the propellants were observed using the VSC to study their original morphology and optical reactivity.

Target Preparation

Pieces of black fabric (each approximately 40 cm × 30 cm) were used as targets. They were held onto a wooden frame.

Test Firing Procedure

Only one shot was shot on one target. Fabric targets were handled carefully in a way that the GSR are not disturbed.

Test firing was conducted at the indoor firing range at the Government Analyst's Department under controlled conditions. The gun was clamped and held perpendicular to the target.

VSC Examination

The shot targets were examined using the VSC 8000 (Foster + Freeman). GSR were observed under IR radiation using the spot light between 485-590 nm and the long pass filter at 645 nm. Magnification was kept constant (Magnification=2.16) throughout the experiment, in order to compare the saved images easily.

Results and Discussion

Examination of Firearms

Details of the firearms which were used for the test firing are given in the Table 1.

Table 1: Features of the handguns used for test firing

Firearm	Long barrel revolver	Short barrel revolver	Pistol 1	Pistol 2
Make	Arminius	Arminius	CZ	Norinco
Model	Details not available		85 B	NP 22
Manufactured country	Germany	Germany	Czech Republic	China
Calibre	.38 special	.38 special	9×19mm	9×19mm
Total length	22.5 cm	18.0 cm	20.5 cm	19.0 cm
Barrel Length	7.5 cm	4.0 cm	11.3 cm	10.8 cm
Weight	835 g	745 g	978 g	880 g

Examination of Ammunitions and their Unburnt Propellants

Ammunitions which were used for the test firing are given in Figure 1.

Information about the ammunition used, and their propellants is given in the Table 2.

Figure 1A: .38 Special cartridge

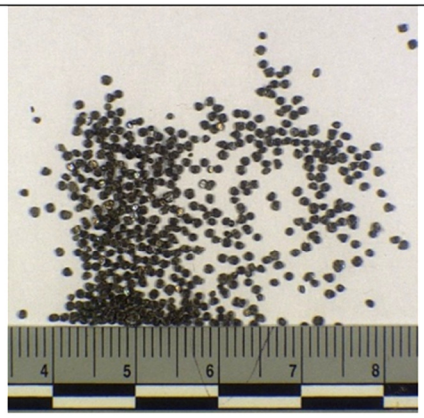
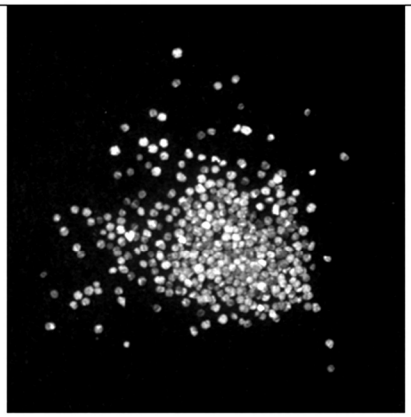
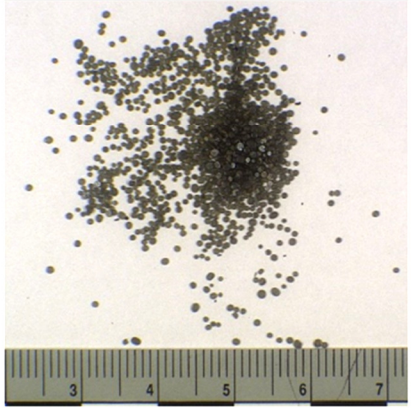
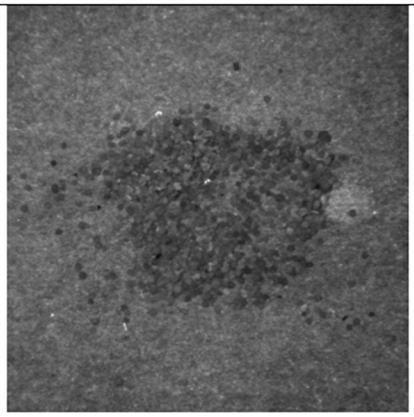
Figure 1B: 9×19mm cartridge

Table 2: Morphology and the optical reactivity of the ammunitions used

Ammunition Calibre	Manufacturer	Head stamp	Morphology of the propellant	Optical Activity of the propellant
.38 Special	PMC, South Korea		Flattened/ Spherical	Yes
9×19mm	PRC		Flattened/ Flakes	No

When unburnt propellants of two types of ammunitions were observed using the VSC, only the unburnt propellant of the .38 Special cartridge showed luminescence, therefore had been active for IR radiation. Unburnt propellants of 9×19mm were IR inactive. Images of the propellants, observed by the VSC under visible and IR radiation are shown in Table 3.

Table 3: VSC Images of propellants, under visible and IR radiation (Magnification=4.3)

Ammunition	Under visible light	Under IR radiation
.38 Special		
9×19mm		

Effect of the radiation type on GSR visualisation

VSC images of the target shots at 10 cm distance caused by the Arminius Long barrel revolver under the visible radiation and IR radiation are given in the Table 4.

Table 4: VSC images of gunshot damages (10 cm distance) by Arminius Long barrel revolver, under visible and IR radiation.

Visible light	IR radiation
	

Unburnt or partially burnt propellant particles cannot be clearly seen under the visible light, while they could be clearly seen under the IR radiation due to luminescence. This implies that the VSC under the IR radiation can be used to visualize the GSR in dark fabrics.

Effect of the Type of the Firearm on the GSR Distribution

VSC images of the GSR distribution of the 10 cm shots carried out using four firearms are given in the Table 5. The GSR luminescent patterns obtained from the tested revolvers were dense and concentric. The GSR luminescent patterns obtained from the tested pistols were found to be more homogenous than those of the revolvers. Although the same type of ammunition was used for both CZ and NP22 pistols, the luminescent particle distribution of the CZ pistol was wider and lesser dense than for the NP22 pistol.

Table 5: VSC images of GSR distribution of the 10 cm distance shots of four handguns

 Arminius Long barrel revolver	 Arminius short barrel revolver
 CZ Pistol	 NP 22 Pistol

It was observed that the appearance and the morphology of the unburnt or partially burnt particles were different from each other depending on the ammunition type. The unburnt or partially burnt particles of 9×19mm cartridges were yellow and black. Though these particles were not studied separately for their composition in this

study, according to previous studies of Rolf Hofer et al. [3], it can be assumed that the black coloured particles were the graphite coated unburnt propellants and the yellow-green particles were the partially burnt propellants of which the graphite layer has been removed during the firing process, which cause luminescence when observed under IR radiation. This could be the reason for the propellants which were not optically active before, being optically active after shooting.

The two types of revolvers which were used are of similar make, the only difference being the length of the barrel. As to the results of this study, for the two Arminius revolvers used, the length of the barrel had no significantly affect to the GSR distribution pattern. It should be noted that the length difference of the two revolvers was 3.5 cm only. In addition, though the same type of ammunition was used for both CZ and NP 22 pistols, the GSR distributions from these pistols were different, demonstrating that the GSR distribution varies with the make of the firearm. Taken together, the above results clearly indicate that the GSR distribution depends on the type of the firearm.

The Effect of Distance on the GSR Distribution

VSC images of the GSR distribution caused by Arminius Long barrel revolver and the CZ Pistol at distances of 10 cm, 20 cm and 40 cm, where the GSR distribution is significant are given in the Table 6 and GSR distribution caused by both hand guns at 80 cm and 120 cm distances are given in the Table 7.

Table 6: VSC images of GSR caused by the Arminius Long barrelled revolver and the CZ Pistol at three distances (10 cm, 20 cm and 40 cm)

Distance / cm	Arminius Long barrel revolver	CZ Pistol
10		
20		
40		

Arminius Long barrel revolver

The luminescent GSR distribution indicates that the density of the GSR particles reduces with the distance. Concentric pattern of GSR distribution could be observed on the target at 10 cm and 20 cm. The density was lower and the distribution was wider in the 20 cm shot. Homogeneous GSR distribution could be observed on targets at 40 cm and 80 cm. The density was lower and the distribution was wider in 80 cm shot. At 120 cm distance, only few luminescent particles were seen.

Table 7: VSC images of GSR caused by the Arminius Long barrelled revolver and the CZ Pistol at two distances (80 cm, 120 cm)

Distance / cm	Arminius Long barrel revolver	CZ Pistol
80		
120		

CZ Pistol

The luminescent GSR particles showed a comparatively more homogeneous distribution on targets at 10 cm, 20 cm and 40 cm distances. With increasing distance, the distribution pattern increased in diameter and the particle density decreased. Few particles were seen at 80 cm and 120 cm distances.

At 40 cm the GSR distribution was homogenous for both types of guns. Accordingly, at 10 cm and 20 cm distances, the revolver and the pistol could be differentiated based on the GSR distribution. More studies need to be carried out using more revolvers and pistols to confirm whether this pattern remains the same.

Conclusion

GSR plays an important role in the firearms related crimes. VSC can be used to observe the GSR under IR radiation. When observed under IR radiation, unburnt or partially burnt particles give fluorescence and provide required contrast to be visible on dark fabrics.

Out of two types of cartridges used in this study, propellants of one cartridge (.38 Special) had luminescent properties before shooting, but after shooting, partially burnt particles of both types of cartridges showed luminescent properties. Effect of the radiation type on GSR visualization study revealed that the GSR on black fabrics are best seen under IR radiation.

Effect of the firearm on the GSR distribution study results revealed that the GSR distribution depends on the type of the firearm. Resultant GSR distributions of the two revolvers used are more concentric than those of the two pistols used. Furthermore, the results of the two pistols used in the study indicate that the GSR distribution is also affected by the make of the firearm.

Effect of the distance on the GSR study results revealed that the GSR distribution varies with the distance regardless of the firearm. The revolver had given a concentric GSR distribution up to 20 cm distance, homogenous distribution up to 80 cm distance. The pistol had given a homogenous distribution up to 40 cm. In this study, the GSR distribution could only be significantly identified up to the distance of 80 cm for the Arminius long barrel revolver and up to 40 cm for the CZ pistol.

Only qualitative comparison of GSR distribution patterns was performed in this study. Future work could include quantitative analysis of particle densities, as demonstrated by Rolf Hofer et al. [3], to provide more objective comparisons of GSR patterns.

It should be stated that use of the VSC is not a confirmation method for GSR detection, but only aids in the detection and visualization.

Reference:

1. *Charles S, Geusens N, Vergalito E, Nys B. 19th INTERPOL International Forensic Science Managers Symposium. Lyon, France: s.n.; 2019. p. 39-67.*
2. *Miller MT. Visualization and Enhancement: Chemical Evidence. Crime Scene Investigation Laboratory Manual. 2018. p. 109-19.*
3. *Hofer R, Graf S, Christen S. The use of unburned propellant powder for shooting-distance determination. Part I: Infrared luminescence. Forensic Sci Int. 2017;10-19.*
4. *López-López M, García-Ruiz C. Recent non-chemical approaches to estimate the shooting distance. Forensic Sci Int. 2014;79-85.*
5. *Carroll MS. The Medical Examiner-Coroner and the Firearms Examiner. In: Rogers CB, Sathyavagiswaran L, editors. Multidisciplinary Medico-Legal Death Investigation Role of Consultants. 2018. p. 245-64.*
6. *Mohd Zain Z, Jaluddin SN, Abdul Halim MI, Mohamed Subri MS. The effect of type of firearm and shooting distance on pattern distribution, particle dispersion and amount of gunshot residue. Egypt J Forensic Sci. 2021.*
7. *Atwater CS, Durina ME, Durina JP, Blackledge RD. Visualization of Gunshot Residue Patterns on Dark Clothing†. J Forensic Sci. 2006.*
8. Bailey JA. Digital infrared photography to develop GSR patternsFootnote. Aust J Forensic Sci. 2007;33-40.

Identification of Kerosene in Fire Debris Evidence in the Presence of Interference from Polypropylene Based Household Products

*Mrs Nawgalage Induma Kalpani Fernando[1], Mr Stephen Paul Andrews[2]
[1] Explosives & Fire Investigation Section, Government Analyst's Department, Sri Lanka
[2]School of Engineering, University of Lancashire, U.K.
*Email: indu.fernandogad@gmail.com

Abstract

The aim of this research was to identify interference in fire debris containing polypropylene (PP) based household products. To achieve this aim, three types of interferences emitted from substrates consisting of selected clean polymer products/items were analyzed, namely interferences from before burning (substrate background products), when heated (pyrolysis products) and after burning (combustion products). The principal objective of this study is to interpret data/chromatograms for the detection of trace levels of kerosene when the chromatograms are dominated by interfering compounds from fire debris containing PP polymeric materials, in samples from real kerosene accelerated fire scenes.

ASTM E-1412 extraction procedure was used for the sample concentration and ASTM E-1618 standard test method was used for the Gas Chromatography/Mass Spectrometry (GC/MS) instrumental data analysis/ interpretation.

PP interfering compounds observed included 2,4-dimethylheptene, C12 and C15 branched alkenes in unheated, heated or pyrolyzed and burnt samples. Kerosene (paraffin) target compounds were consecutive chain hydrocarbons of nonane (C9) – heptadecane (C17). Even though these PP interfering compounds were not affecting identification, they resulted in masked peaks and false negatives for lower concentrations of kerosene. It was found that concentrations greater than five times that of the limit of detection (LOD) for neat kerosene could be detected while concentrations lower than those levels were accounted as negative for kerosene.

Introduction

Fire debris contain many interferences that make it difficult to interpret the obtained results [1]. Compared to research that did not include spiking tests, and even if included, significant amounts of petrol were used and unburnt clean samples were not tested for comparisons [2], this paper identifies interferences in fire debris containing two commonly found polypropylene (PP) based household products. Further, this study consists of the identification of kerosene known as paraffin in the United Kingdom as the fire accelerant in the presence of PP interference.

Method consisted of analyzing samples for interfering compounds; these samples were neat/pure unheated test samples, heated samples for pyrolysis compounds, burnt samples spiked with kerosene and without kerosene (burnt in open air). As a further experiment, pyrolyzed polymer sample extracts were spiked with known amounts of kerosene to understand the LOD. Solvent extraction using passive headspace adsorption by charcoal strips followed by diethyl ether was conducted using ASTM E-1412 standard practice [3]. Analysis using GC/MS and data interpretation was done according to the ASTM E-1618[4] standard test method.

The interfering compounds in PP were identified as 2,4-dimethylheptene, C12 and C15 branched alkenes as stated in the literature [2,5].

Target compounds starting from C9-C17 straight-chain alkanes could be identified for this reference kerosene (RK), consistent with the kerosenes mentioned in the literature [4,6]. This kerosene is classified under heavy petroleum distillate (HPD) by the ASTM E1618-19 guidelines [4], and this particular brand obtained from Bartoline Ltd, UK not containing any aromatic components and cyclohexanes posed a challenge for the detection of trace levels of kerosene in the presence of any interfering compound. However, as a result, this kerosene brand gave an additional opportunity to test its detection limit when it was mixed with known amounts of pyrolyzed extracts of PP.

According to the results obtained, interpretation was straightforward for all fresh kerosene spiked on burnt PP samples, since all interfering peaks had been masked by kerosene peaks which were more prominent. When pyrolysate was tested with known amounts of kerosene to study the masking behavior of PP, it was found that LOD was nearly five times greater than added kerosene. Therefore, it was certain that PP causes a false negative identification for traces of kerosene.

Research methodology

Chemicals

Diethyl ether, HPLC grade (Fisher Scientific) was used as the solvent for the extraction procedure, solvent for blank injections, and the solvent for dilution of neat kerosene.

Reference kerosene (paraffin) sample was prepared by using neat paraffin (can of 4 L) obtained from Bartoline Ltd, UK.

Materials

Unlined metal cans from BIRMINGHAM TIN BOX, INCORPORATING BTB PACKAGING, UK were used for the burning of samples and for the heated headspace extraction of fire debris samples. Activated charcoal strips (20 mm x 8 mm x 1 mm) obtained from ARROWHEAD FORENSICS, US were used for passive heated headspace extraction. Uncontaminated paper clips and white twines were used to suspend the carbon strips. Small container boxes (white in color) and disposable clear cups which are two common household products made of PP were selected as test samples.

Instruments and equipment

STUART SCIENTIFIC, SA3 vortex mixer, model VWR DRY-Line DL 53, DL 115 oven, model VWR top loading balance, micro pipettes of 10 µl (Labnet), 10-100 µl (Accumax) and 1000 µl (Accumax) were used.

GC- Thermo Scientific Trace 1300 Series Ultra Gas Chromatograph coupled with MS- ISQ_{LT} Single Quadrupole Mass Spectrometer. Type of the column equipped with the GC was Rxi®-5ms having 30 m of length, 0.25mm of internal diameter and 0.25 µm of film thickness and helium was used as the carrier gas (1.5 ml/min). Thermo SCIENTIFIC TriPlus 100 LS auto sampler having 162 sites for sample vials and Thermo Xcalibur software enabled searching identity of components by NIST (National Institute of Standards and Technology) library were used for the analyses.

Figure 1: Clean (uncontaminated) container boxes and PP disposable cups.

Method

A diluted series of neat kerosene of 0.1%, 0.01% and 0.005% were prepared respectively using diethyl ether to determine the LOD of reference kerosene (RK) for the instrument.

PP samples (10 g) were kept in metal cans and labelled properly (Figure 01) by categorizing into four groups as samples for substrate interfering compounds (unheated), pyrolysis (heating by modified destructive distillation method[7] – Figure 2), burning in open air after spiking with fresh kerosene (2.00 ml) and without kerosene (Figure 3).

Figure 2: Pyrolysis/heating of polymer samples.

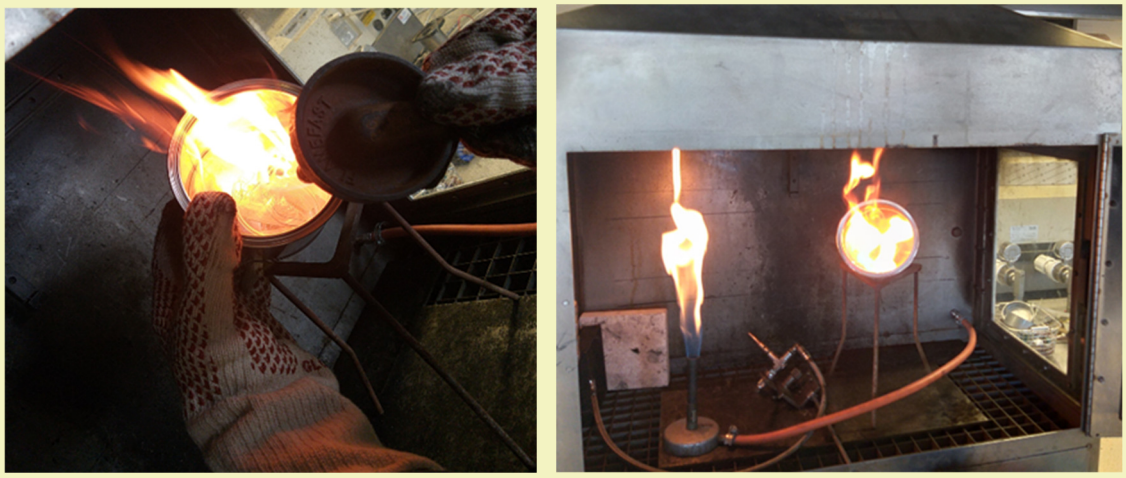


Figure 3: Burning of polymer samples.

Interfering products from above mentioned four categories of samples and kerosene residues from kerosene spiked burnt samples were adsorbed onto activated charcoal strips by heated passive headspace technique by oven heating at 80 ^{0}C for 08 hours. Then volatile compounds of activated charcoal strips were desorbed into diethyl ether for instrumental analysis.

By changing the volume of the pyrolyzed extract and volume of the 0.1 % RK, different mixtures were created to study masking performance by PP and determine LOD of kerosene in the presence of PP interfering compounds. All above samples' analyses were done using the GC/MS according to the temperature program given in Table 1.

Table 1: GC Temperature Program

Initial temperature	50 ^{0}C
Initial hold time	2 min
Ramp rate	15 ^{0}C / min
Final temperature	250 ^{0}C
Total run time	15.5 min

Temperature of the ion source of mass spectrometer was 230 ^{0}C and mass transfer line temperature was 250 ^{0}C. Mass scan range was selected as 33 to 550 amu and mass spectra were obtained in full-scan mode.

Data interpretation was done mainly by visual pattern recognition of total ion chromatograms (TICs) with compared to 0.1 % RK. Further chromatographic data analysis was done using extracted ion profiling (EIP) and target compound analysis or both where relevant. Identification of target compounds was done with the NIST library and verifications were recorded when their matching scores were good enough.

Results and discussion

Determination of instrumental limit of detection (LOD) for RK or fresh kerosene

When the concentration of RK was 0.1%, consecutive alkanes from nonane to pentadecane were detected between 3.78 min - 9.80 min as shown in Figure 4 (first TIC from the top in black). Peaks which appeared after the prominent peak of tridecane at 8.09 min were tetradecane and pentadecane. Their peak heights and relative abundance were small compared to other peaks at the lighter end of the chromatogram. When considering concentration of RK of 0.01%, it was not possible to detect these tetradecane and pentadecane peaks from the TIC. But, its EIC had resolved and could be used to visualized both low abundance tetradecane and pentadecane peaks. When the concentration of kerosene was further decreased to 0.005%, both TIC and EIP showed tridecane peak well since it was the most abundant constituent in kerosene and did not show tetradecane and tridecane peaks.

Therefore, LOD of neat RK was determined as 0.01 % in the instrument for this study and that level was also considered when investigating the LOD for spiked kerosene in the presence of interfering compounds from PP.

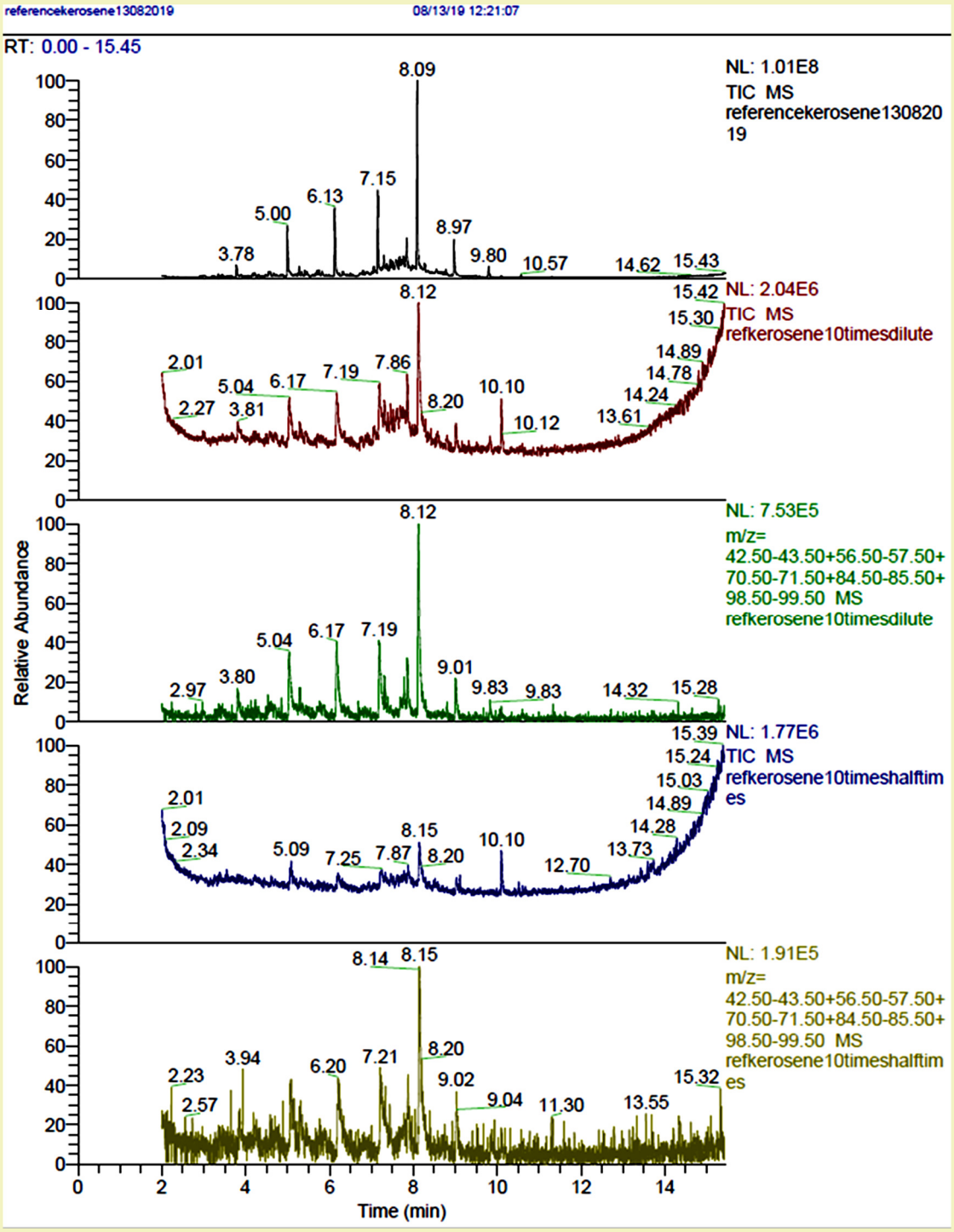


Figure 4: (From the top)
TIC for 0.1 %, 0.01 % and its EIP, 0.005 % and its EIP of diluted reference kerosene

Identification of PP interfering compounds and interpretation of chromatograms dominated with kerosene

TICs for clean unburnt PP products tested

TICs from unburnt clean PP container boxes, disposable cups and RK for comparison are shown in Figure 5. Target compounds from each product are given in Table 2. Hexane, 2, 3, 5-trimethyl and octane, 4-methyl eluted at 2.80 min and 3.30 min respectively for the unburnt clean PP samples. Here, C_{12} branched alkenes and C_{15} branched alkenes were dominant and only PP cups showed 2,4-dimethylheptene.

Pattern recognition and target compounds analysis did not match with kerosene and hence it was clear that there was no chance to misinterpret PP interferences as kerosene.

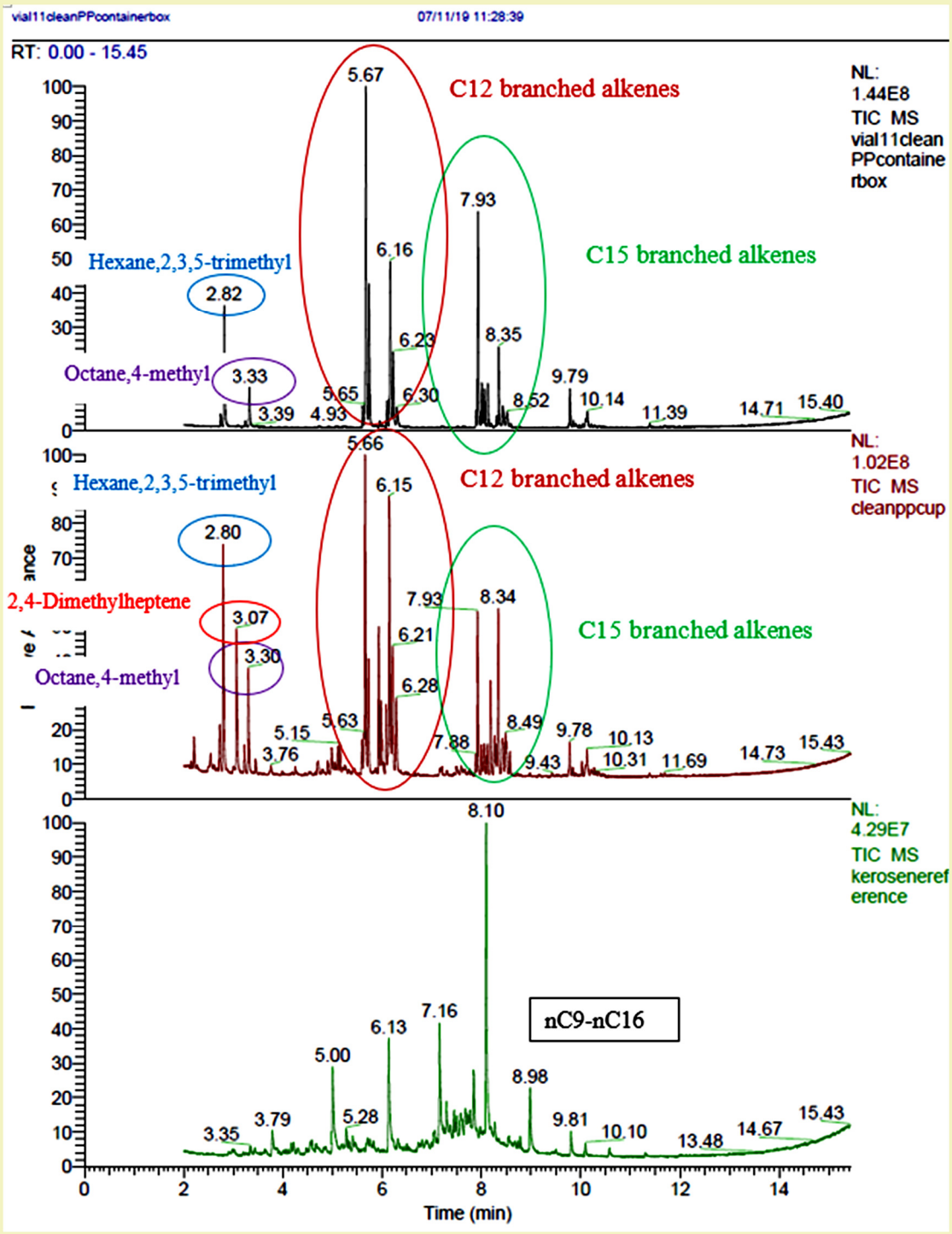


Figure 5: (From the top) TIC for clean PP container boxes, clean PP cups and RK

Table 2: Identified target compounds in clean unburnt PP products tested

PP (boxes) /RtT/min	PP (cups) /RtT/min	Target compound
2.81	2.80	Hexane,2,3,5-trimethyl
-	3.07	2,4-Dimethylheptene
3.33	3.30	Octane,4-methyl
5.65-6.30	5.63-6.28	C12 branched alkenes
7.93-8.52	7.88-8.49	C15 branched alkenes

TICs for pyrolyzed and burnt PP products

Chromatograms (Figure 6 and 7) for all these pyrolyzed and burnt samples exhibited reproducible results (Table 3) for the two items tested. Here, 2,4-dimethylheptene, C12 and C15 branched alkenes eluted as stated in the literature [4,5].

Both target compounds matching and pattern matching for heated and burnt samples of PP did not fit for kerosene identification and there was no possibility to misinterpret as kerosene.

Since kerosene peaks had shielded all these interfering peaks in chromatograms obtained for kerosene spiked samples (second TIC from the top in both 6 and 7 Figures), their interpretation was straightforward.

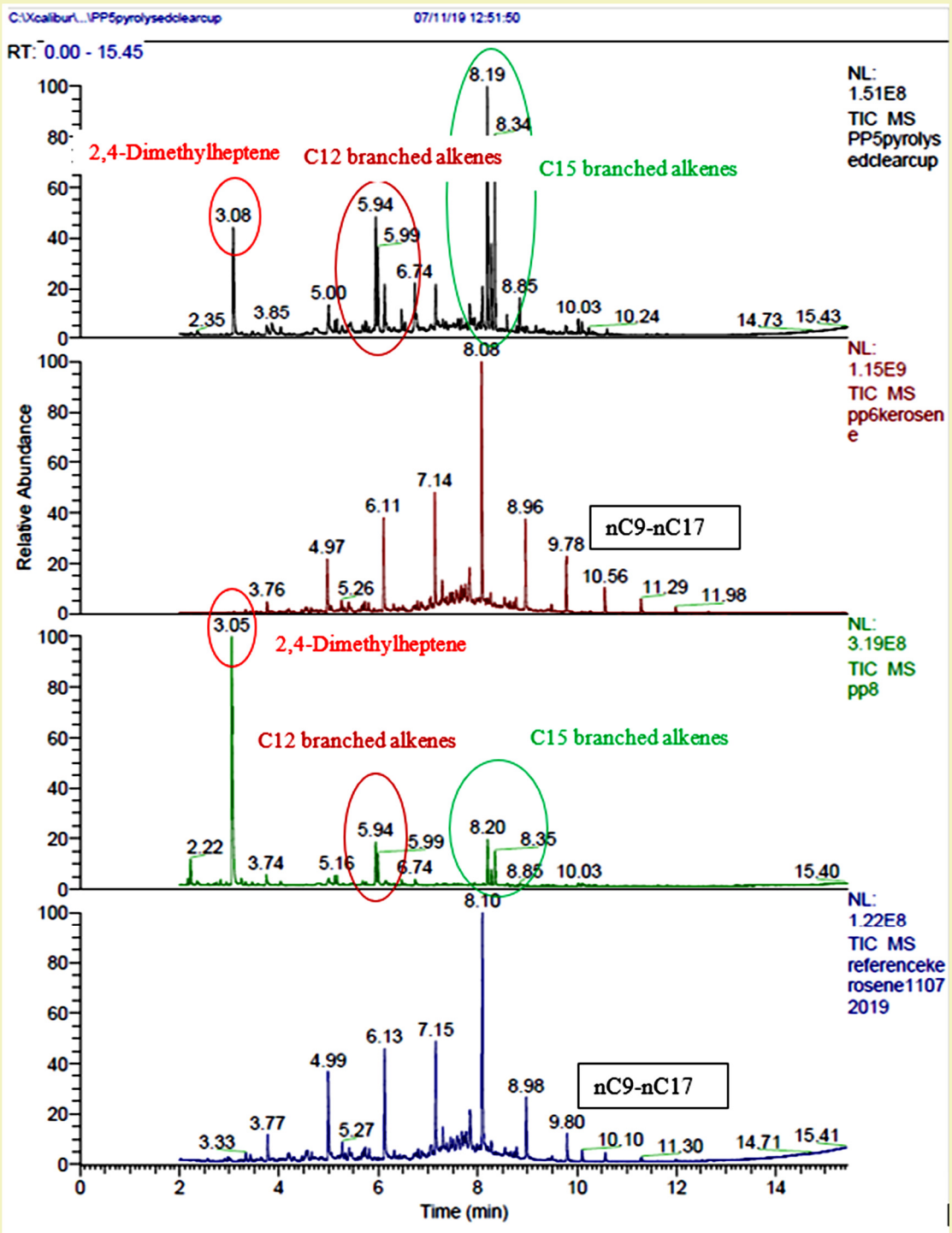


Figure 6: (PP container boxes– from the top)
TIC for sample of pyrolyzed product, burnt with kerosene, burnt without kerosene and RK

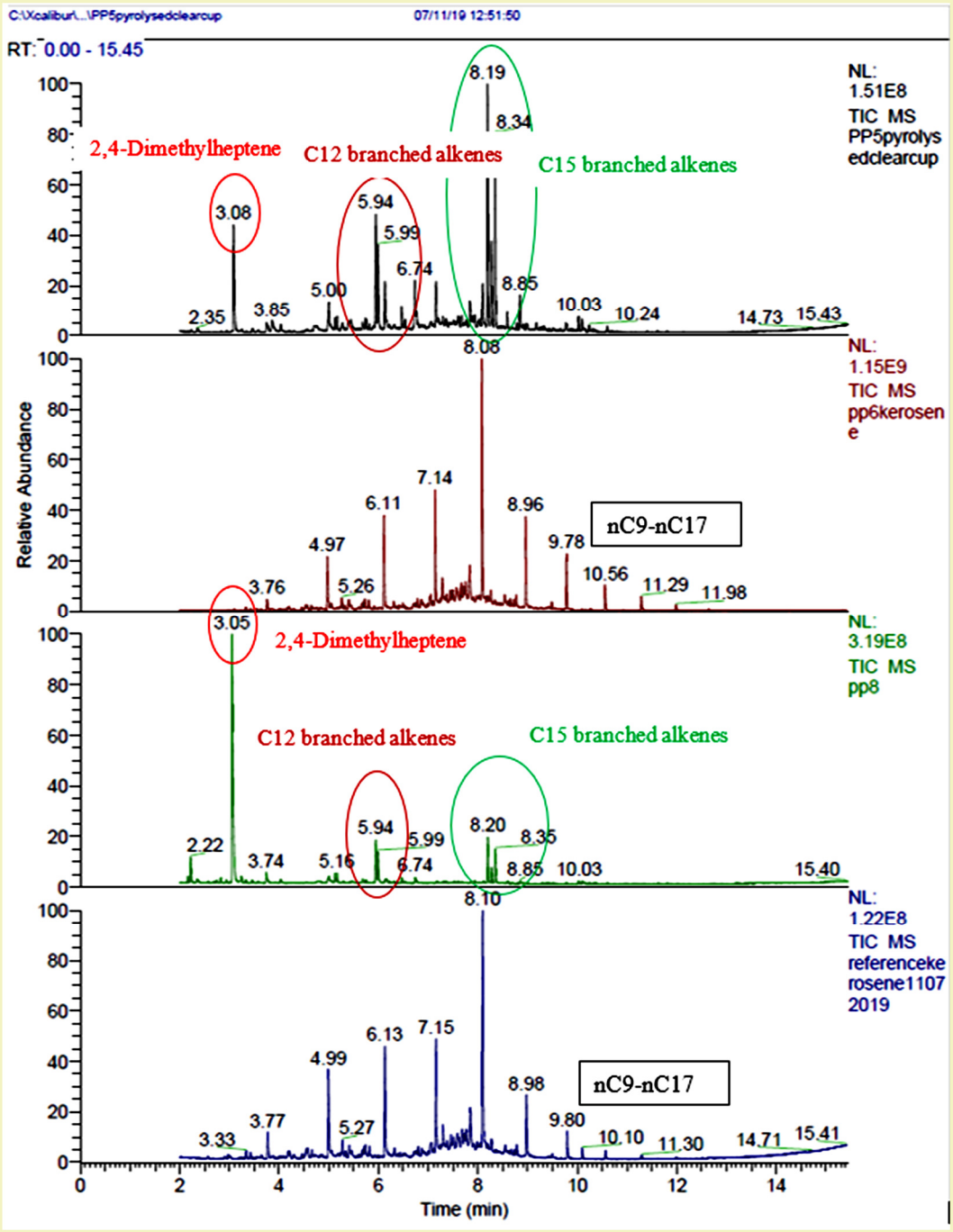


Figure 7: (PP disposable cups– from the top) TIC for sample of pyrolyzed product, burnt with kerosene, burnt without kerosene and RK

Table 3: Identified target compounds in pyrolyzed and burnt PP products

RtT/min, pyrolyzed sample		RtT/min, burnt with kerosene		RtT/min, burnt without kerosene		Target compound
PP1/container boxes	PP5/cups	PP2/container boxes	PP6/cups	PP7/container boxes	PP8/cups	
2.21	-	-	-	2.22	-	4-methylheptene
3.04	3.05	-	-	3.05	3.05	2,4-dimethylheptene
-	-	4.99	4.97	-	-	Decane
-	-	6.11	6.11	-	-	Undecane
-	-	7.14	7.14	-	-	Dodecane
-	-	8.08	8.08	-	-	Tridecane
-	-	8.96	8.96	-	-	Tetradecane
-	-	9.79	9.78	-	-	Pentadecane
-	-	10.56	10.56	-	-	Hexadecane
-	-	-	11.29	-	-	Heptadecane
5.66-5.93	5.94-5.99	-	-	5.68-5.99	5.94-5.99	C12 branched alkenes
7.83-8.34	8.10-8.34	-	-	7.94-8.35	8.20-8.35	C15 branched alkenes

Interpretation of data when known amounts of extracted polymer samples were spiked with known amounts of kerosene

Determination of LOD for kerosene when an extracted sample of PP (container boxes) was spiked with kerosene

When a pyrolyzed extract of PP was spiked with different quantities of kerosene, TICs could be seen as in Figure 8. It is clear that when considering the second TIC from the top in red, even though the kerosene level of the mixture was higher (0.025%) than LOD of kerosene (identified as 0.01% for this brand in this instrument), interfering peaks had shielded the kerosene peaks. When the ratio of the mixture was 1:1, the concentration of kerosene was 0.05% and it started to resolve kerosene peaks as shown in EIP (fourth chromatogram from the top in blue). Therefore, 0.05% (five times greater than instrumental LOD for fresh kerosene) was concluded as LOD for kerosene in the presence of PP interference.

When kerosene was further increased to 150 µl, peaks resolved well (second EIP from the bottom in pink). The concentration of kerosene was 0.075% and it was undoubtedly higher than instrumental LOD.

Therefore, it was an evidence to prove that PP interference can mask trace levels of kerosene and be misinterpreted as negative.

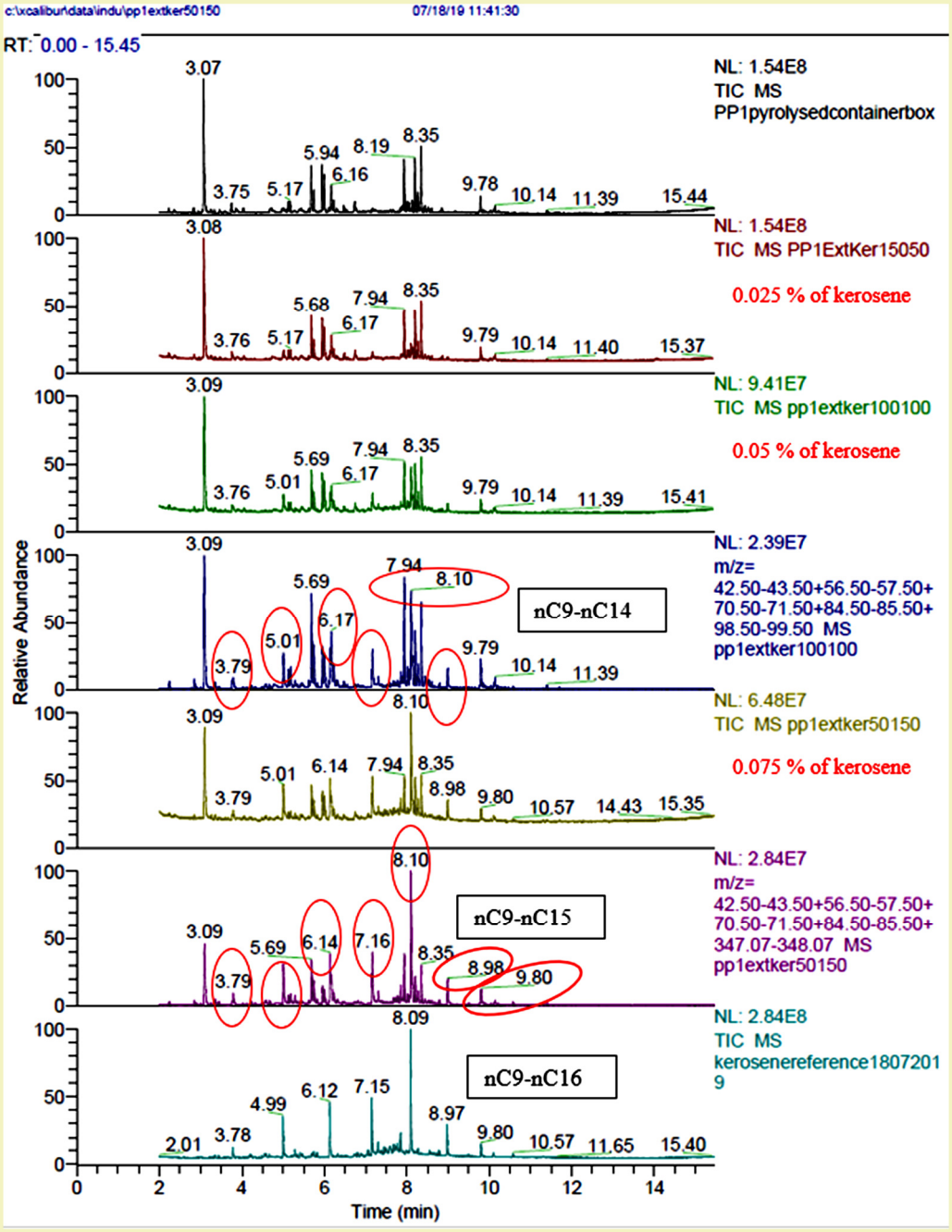


Figure 8: (Pyrolyzed extract of PP container boxes- From the top) TIC for extract, TIC for mixture of extract: kerosene-150:50, 100:100 and its EIP, TIC for 50:150 and its EIP and TIC of RK

Conclusion

PP interfering compounds were found to be 2,4-dimethylheptene, C12 and C15 branched alkenes.

Interpretation of data was straightforward for kerosene-spiked burnt PP samples since these chromatographic patterns were quite different and interfering compounds did not fit for target compounds in kerosene.

However, these interferences masked the trace levels of kerosene and when it was examined for the presence of known kerosene in pyrolyzed extract, the LOD was accounted to be nearly five times greater than for fresh kerosene. It might cause a false negative identification.

References:

1. *Stauffer E, Dolan JA, Newman R. Fire Debris Analysis. Academic Press; 2008.*
2. *Stauffer, E. (2003) Concept of pyrolysis for fire debris analysts; Science & Justice, 43(1), pp. 29-40. doi: 10.1016/S1355-0306(03)71738-9.*
3. *ASTM International (2016) E1412-16: Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration with Activated Charcoal.*
4. *ASTM International (2014) E1618-19: Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry.*
5. *Hendrikse, J., Grutters, M. and Schäfer, F. (2016) Identifying ignitable liquids in fire debris: a guideline for forensic experts. Amsterdam, Netherlands: Academic Press.*
6. *Castelbuono, J. (2008) The identification of ignitable liquids in the presence of pyrolysis products: Generation of a pyrolysis product database. M.Sc. Thesis, University of Central Florida. [online] Available at: http://purl.fcla.edu/fcla/etd/CFE0002429 (Accessed on 05.06.2024).*
7. *National Center for Forensic Science (2006) Substrate Materials Sample Preparation. [online] Available at: http://ilrc.ucf.edu/substrate/criteria.php (Accessed on 21.08.2024).*

Evaluation of DNA Extraction Efficiency based on Magnetic Bead Technology between Semi-Automated and Manual Methods for Bone and Tooth Samples in Forensic Science

Mr. Bao Vuong Gia, Mrs. Binh Nguyen Ngoc Hoa, Mrs. Trang Nguyen Huynh Minh, Ms. Huong Nguyen Thi Lan**
Forensic Medicine Center of Ho Chi Minh City, Ho Chi Minh City, Vietnam
*Email: *giabaovuong10@gmail.com, **ntlhuong226@gmail.com*

Abstract

Identification from bone and tooth samples is a crucial aspect of forensic science, aiding in criminal investigations and the identification of missing persons. However, DNA extraction from skeletal remains presents significant challenges due to degradation, environmental exposure, and PCR inhibitors. Optimizing extraction methods is essential to improve DNA recovery efficiency and forensic analysis. This study compared two DNA extraction methods, manual and semi-automated, on 36 bone and tooth samples from unidentified remains. DNA was extracted using the Bone DNA Extraction Kit (Promega) through two approaches: manual extraction and semi-automated extraction with the Maxwell® RSC 16 system. The median DNA concentration obtained from the manual extraction method (2740 ng/mL) was significantly higher than that of the semi-automated method (1975 ng/mL) with $p = 0.0024$. However, both methods achieved an equivalent success rate of full DNA profiles (24/36 samples, 66.67%). Although the manual extraction method yielded higher DNA concentrations, the semi-automated approach demonstrated comparable efficiency in DNA profiling while reducing processing time and contamination risks. Semi-automated systems can be a promising option for large-scale forensic applications, despite their higher per-sample cost. Further research is needed to optimize automated workflows to enhance DNA recovery from highly degraded skeletal remains.

Keywords: semi-automated extraction, manual extraction, forensic science, magnetic bead

Introduction

Identification from remains is an important requirement in forensic science, serving many purposes such as criminal investigation, identifying missing persons. The increasing number of unsolved cases, prolonged disappearances and the growing need for identification make research and improvement of DNA extraction methods from remains not only a scientific need but also an urgent social requirement.

Unlike blood samples, buccal swab cells or soft tissue, old remains are often degenerated, making DNA extraction difficult. DNA can be severely degraded by environmental factors such as temperature, humidity, bacteria and chemicals in the soil. This affects the ability to amplify and type genetic profile. In addition, bones contain many PCR inhibitors such as humic acid, tannin and collagen, which reduce the analytical efficiency. A factor for successful typing is the DNA extraction protocol, which plays a key role in the quality and quantity of DNA obtained and amplification success [1,2]. A good DNA extraction protocol should be robust, effective at recovering DNA and removing inhibitors, non-toxic, cost-effective, rapid and simple. Traditional methods such as phenol-chloroform are both time-consuming and potentially pose a risk of toxic exposure. These challenges require optimization and automation of the DNA extraction process to improve the efficiency of DNA recovery from bone and tooth samples under severe degradation conditions.

With the rapid development of biotechnology, many automated DNA extraction systems have been developed to reduce analysis time, increase DNA recovery efficiency and limit the risk of contamination. Studies have shown that automating the DNA extraction process from remains is an inevitable trend. For example, Zupanič et al (2016) optimized the DNA extraction process from human remains using the AutoMate Express system and the PrepFiler BTA kit, which increased the rate of obtaining complete DNA profiles [3]. Duijs and Sijen (2020) developed a semi-automated method for DNA extraction that is faster than traditional methods [4]. Zoranjic et al (2021) described that the PrepFiler Express BTA™ method outperformed organic methods in DNA recovery from bones and teeth [5].

These studies show that without improvements in DNA extraction processes, DNA identification from human remains will still face many limitations in terms of time, quality, and accuracy. Therefore, research and application of advanced DNA extraction methods is not only a scientific need but also an urgent requirement to improve the efficiency of forensic examination and resolve pending cases.

Materials and Methods

Materials

A total of 36 samples (2 tibia, 1 phalanx, 1 radius, 13 femur samples and 19 tooth samples) were collected from unidentified human skeletal remains for personal identification in the Forensic Medicine Center of Ho Chi Minh City. The individuals were mostly adults (18-50 years old). Although the samples were found in a variety of locations (soaked in streams, buried in soil, lying on grass), most of them were still solid (Figure 1).

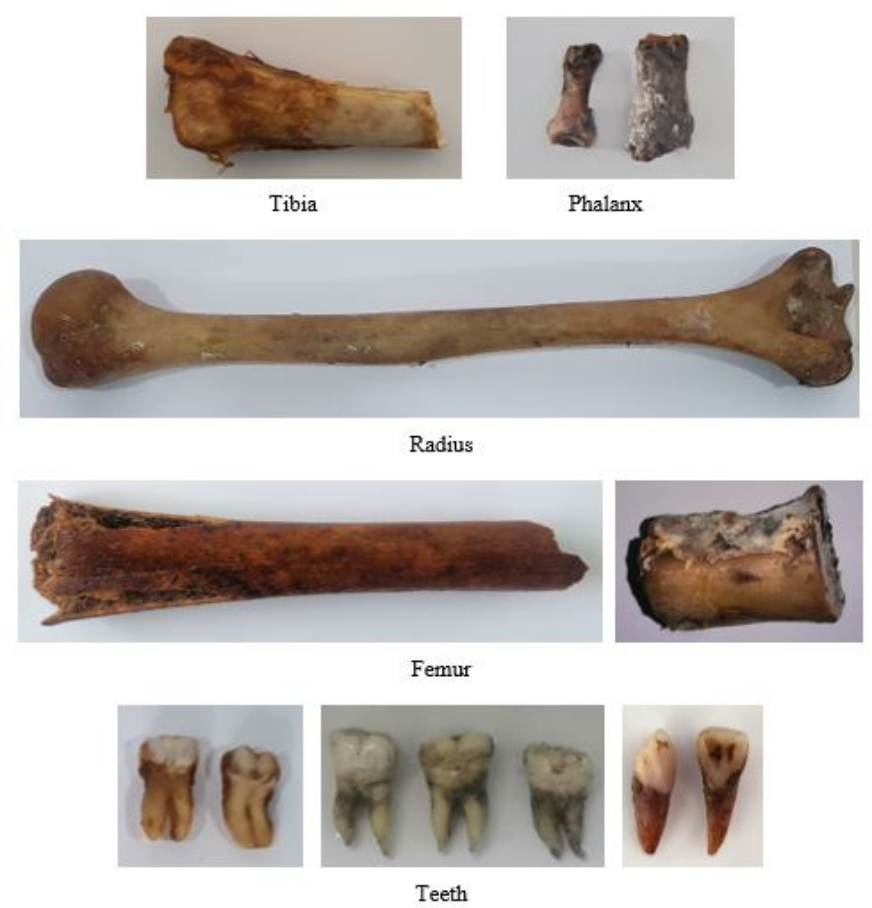


Figure 1: Bone and tooth samples.

Methods

Sample treatment:

The most solid and rigid part or structure of the bones and teeth was identified. The selected samples were cleaned with a 5% NaClO solution (Sigma, Germany) and then rinsed with distilled water to remove NaClO. The samples were left to dry, and ground into a fine powder using a mixing mill machine (Retsch MM400, Germany).

DNA extraction:

100 mg of bone/tooth powder was pre-treated by using the Bone DNA Extraction kit (Promega, USA) according to the manufacturer's (Promega) instructions. Each sample was added 400 µL lysis cocktail A in a 1.5 mL microcentrifuge tube, and vortexed for at least 10s. Then, samples were incubated for 2.5h at 56°C while shaking in a thermomixer at 1000 rpm. Full separation of bone/tooth powder residues and lysate is accomplished by centrifuging for 5 minutes at 13000 rpm. 800µl of bone lysis cocktail B was added to each 1.5ml microcentrifuge tube containing 300 µL lysate and mixed carefully. For manual extraction, refer to Bone DNA Extraction Kit: Instructions for Use of Product #TM691 (pp. 5). For semi-automated extraction, the total volume (1.1 ml) is transferred to the Maxwell cartridge and DNA was extracted using the Maxwell® FSC DNA IQ™ Casework Kit (Promega) on the Maxwell® RSC 16 Instrument (Promega) according to the manufacturer's instruction. For extraction, an elution volume of 100 µL Elution Buffer (Promega) was used; then each DNA solution was concentrated by vacuum cleaner into 40 µL.

DNA quantification:

The concentration of extracted DNA was quantified by using the Qubit 2.0 fluorometer (Thermo Fisher Scientific, USA) and the dsDNA Quantification High Sensitivity Kit (BioDynami, USA), following the manufacturer's recommended protocols. For each sample, 2 µl of the extracted DNA was mixed with 198 µl of the working solution before measurement.

Amplification by PCR:

The DNA samples were amplified through PCR using the PowerPlex® Fusion System kit (Promega - USA). For PCR, 7.5 µl DNA solution, 5 µL PowerPlex® Fusion 5X Master Mix, 5 µL PowerPlex® Fusion 5X Primer Pair Mix and 7.5 µL water were used. The optimum DNA concentration for amplification is 1ng/µL. Samples with less than ideal DNA were concentrated, using 15 µL of DNA solution and adding no water to the PCR mix, samples with DNA above the ideal were diluted in sterile water. The amplification was performed on a Veriti™ 96-well thermal cycler (Thermo Fisher Scientific, USA). The reaction set-up and thermal cycling conditions were performed in accordance with the manufacturer's instructions.

Capillary electrophoresis:

The PCR products were subjected to capillary electrophoresis on ABI 3500 Genetic Analyzer (Applied Biosystems, USA), following manufacturer's recommendations. For the capillary electrophoresis step, 2 µL of DNA plus a solution of 10 µL of a solution containing Hidi-formamide and WEN ILS were used. Data was collected with Data Collection v1.0 software. Electrophoretic results were analyzed using GeneMapper® ID-X software v1.4 (Applied Biosystems, USA).

Statistical analysis:

To compare the yield (extracted DNA yield and the number of established a-STR loci) between the different DNA extraction methods, Median, Wilcoxon Signed Rank test and Fisher test were performed with STATA 17 software.

Results and Discussion

Comparison of DNA yield between the manual and semi-automated DNA extraction methods

The DNA concentration recovered from 36 samples using both extraction methods was illustrated in Figure 2. Samples that were manually extracted displayed a median concentration of 2740 ng/mL. Extracts prepared by the Maxwell instrument showed a median concentration of 1975 ng/mL. The difference in quantity was statistically significant (p = 0.0024). The median of the DNA quantity in the manually isolated samples tends to be larger than in semi-automatically isolated samples (Figure 3).

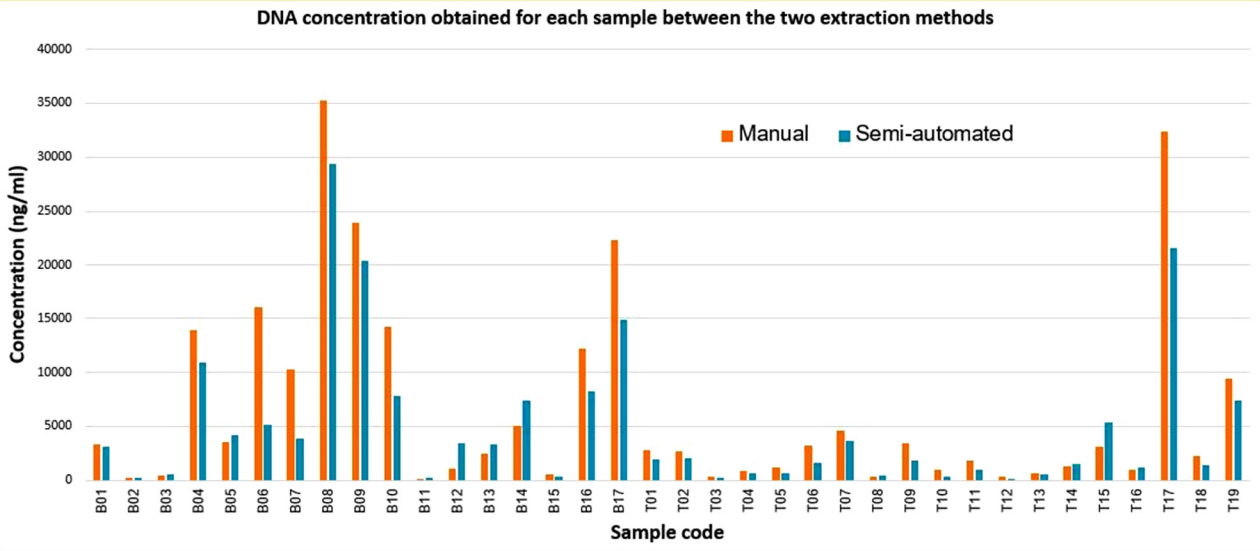


Figure 2: DNA concentration obtained for each sample between the two extraction methods.

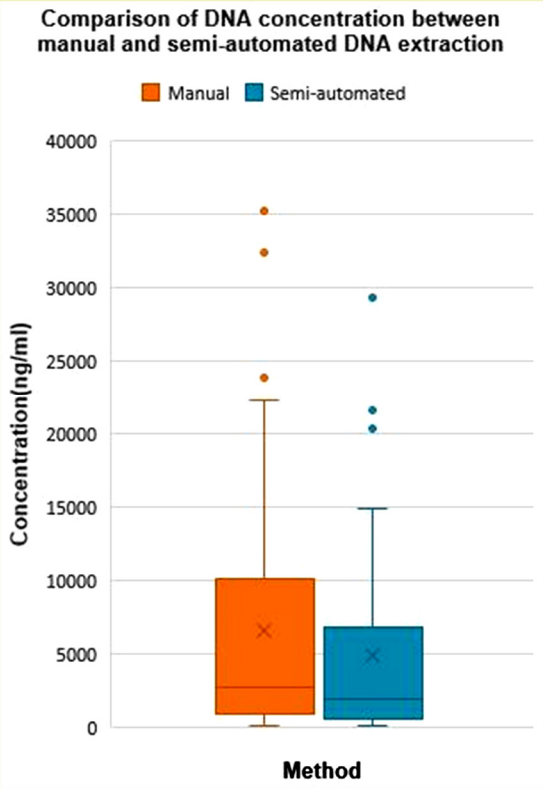


Figure 3: Comparison of DNA concentration between the manual and semi-automated DNA

The routine semi-automated hard tissue sample processing will be a key to efficient sample analysis, potentially replacing manual extraction procedures, reducing hands-on time [6]. However, the comparison of manual and semi-automated DNA extraction processes revealed a slight advantage for manually extracted DNA. The yield of manually prepared DNA was higher than the yield of Maxwell extracts when the procedures were performed according to the supplier's manual. This result is similar to some previous studies [6-9].

DNA profile results

The result of the DNA profiles (number of amplified a-STR loci) was illustrated in Figure 4. The number of loci obtained from both extraction methods was fairly comparable, but some samples (such as B10, B12, T02, and T05) showed significant differences between the two methods. This suggested that DNA extraction efficiency may vary depending on the sample and the method used. Additionally, the number of full a-STR profiles was similar in both extraction methods, with 24 out of 36 samples (66.67%) successfully amplified (Figure 5).

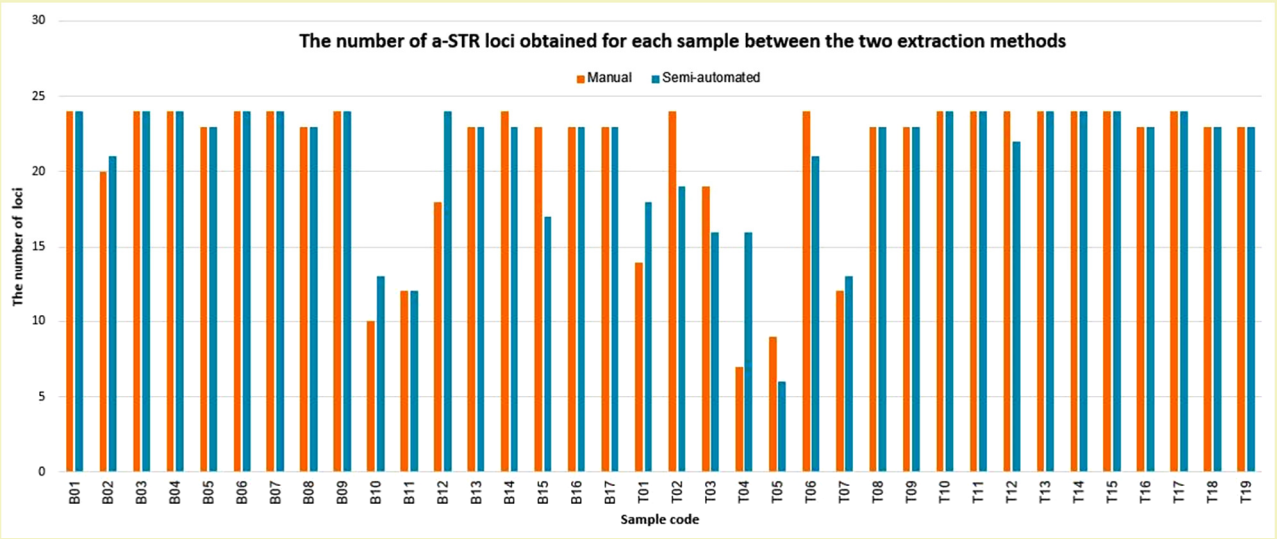


Figure 4: Representative chart of the number of a-STR loci obtained for each sample between the two extraction methods.

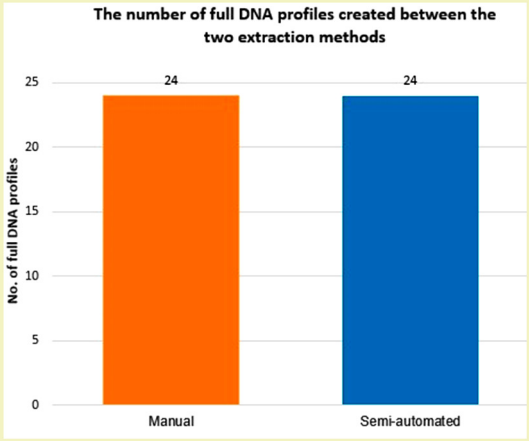


Figure 5: Comparison of the number of full DNA profiles created between the two extraction methods.

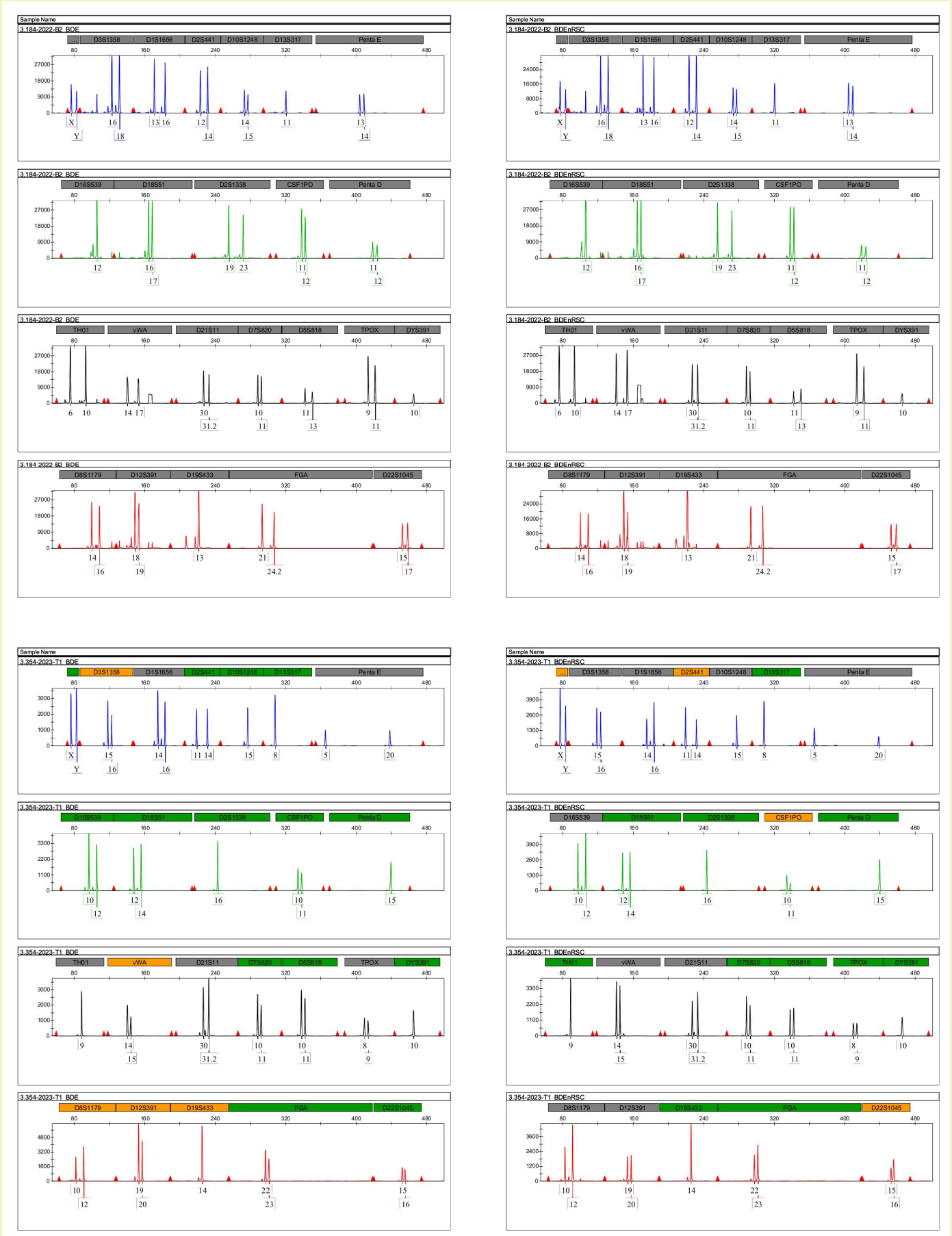


Figure 6: Short tandem repeat profiles of bone and tooth samples generated using the PowerPlex® Fusion System kit between the manual and semi-automated DNA extraction methods. Manual extraction (left) and semi-automated extraction (right) from bone sample (above) and tooth sample (under).

Figure 6 illustrated the DNA profiles of bone and tooth samples obtained between the two extraction methods. Although the DNA concentration from semi-automatically isolated samples was lower than that from manually isolated samples, similar DNA profiles were detected in most of the samples with both methods. This showed the potential application of automated/semi-automated extraction systems in the processing of highly degraded forensic remains.

The manual extraction method, under normal conditions, consumed longer time than the semi-automated extraction method (Table 1). According to the manufacturer's instructions, excluding initial sample incubation time, the semi-automated extraction process took about 25 minutes per run. While manual process could take up to 30-45 minutes depending on the number of samples. With $p = 0.0095$, the difference between the two average time groups (3.81 ± 0.22 hours for manual extraction and 3.73 ± 0.17 hours for semi-automated extraction) was statistically significant ($p < 0.051$). However, the difference was not substantial enough to significantly impact the overall process.

Table 1: DNA profile results by extraction protocols (n=36).

Assessment standards	Manual extraction	Semi-automatic extraction	P value
Mean DNA concentration (ng/ml)	2740	1970	**<0.01[a]**
The completeness level of a-STR profile			
Full profile	24	24	
Large profile (>= 10 loci)	10	11	>0.05[b]
Small profile (< 10 loci)	2	1	
No profile	0	0	
Time range (hour)	3.81 ± 0.22	3.73 ± 0.17	**<0.01[c]**

a: Wilcoxon Signed Rank test, b: Fisher test, c: t-test

Conclusions

The performance of the Maxwell® RSC 16 Instrument was tested on bone and tooth samples, and results were compared with manually isolated samples. Although manual extraction achieved better technical results in DNA concentration, the semi-automated method also provided full DNA profiles similar to the manual method. Less hands -on time and a greater number of samples can be processed at once make the Maxwell® RSC 16 Instrument a potentiallly alternative for larger sample preparations, even though the cost per sample is higher than that for Bone DNA Extraction Kit.

References:

1. *Putkonen MT, Palo JU, Cano JM, Hedman M, Sajantila A. Factors affecting the STR amplification success in poorly preserved bone samples. Investig. Genet. 2010;1(1): 9.*
2. *Irwin JA, Just RS, Loreille OM, Parsons TJ. Characterization of a modified amplification approach for improved STR recovery from severely degraded skeletal elements. Forensic Sci. Int. Genet. 2012;6:578-587.*
3. *Zupanič PI, Debska M, Gornjak PB, et al. Highly efficient automated extraction of DNA from old and contemporary skeletal remains. J. Forensic Leg. Med. 2016,Jan;37:78-86.*
4. *Duijs FE, Sijen T. A rapid and efficient method for DNA extraction from bone powder. Forensic Sci. Int. Rep. 2020;2:100099.*
5. *Zoranjic J, Tay J, Mountford N, Rye M. Optimisation of an Automated DNA Extraction Method for Bone and Teeth Samples and Applicability to Two Forensic Cases. Forensic Sciences. 2021;1:194-201.*
6. *Kalmár A, Péterfia B, Wichmann B, et al. Comparison of Automated and Manual DNA Isolation Methods for DNA Methylation Analysis of Biopsy, Fresh Frozen, and Formalin-Fixed, Paraffin-Embedded Colorectal Cancer Samples. J. Lab. Autom. 2015;20(6):642-651.*
7. *Riemann K, Adamzik M, Frauenrath S, et al. Comparison of manual and automated nucleic acid extraction from whole-blood samples. J. Clin. Lab. Anal. 2007;21(4),244-248.*
8. *Haarkötter C, Gálvez X, Vinueza-Espinosa DC, et al. A comparison of five DNA extraction methods from degraded human skeletal remains. Forensic Sci. Int. 2023,Jul;348:111730.*
9. *Kwa WT, Sim CK, Low A, et al. A Comparison of Three Automated Nucleic Acid Extraction Systems for Human Stool Samples. Microorganisms. 2024;12:2417.*

Technical Article

Identification of Biological Materials of Cattle Using Genetic Markers

Mrs Maftuna Nosirova
Republican Center for Forensic Examination under the Ministry of Justice, Republic of Uzbekistan
Email: maftun.nosirova1990@gmail.com

Abstract

The article describes the organization of conditions for the collection and storage of biological materials of cattle breeds from the population and farms, the necessary equipment and consumables used in the process of DNA identification studies of biological materials obtained from cattle breeds, methods of DNA isolation, information on the amplification of isolated DNA samples , analysis of the results obtained by cattle breeds, identification of biological samples based on the results obtained. Also, based on the fact that the procedure for working with each biological material has its own differences, a more in-depth study of these processes is required.

Keywords: DNA extraction, forensic practice, polymer chain reaction, identification, amplification, interpretation.

Introduction

At the end of the last century, genetic technologies entered human life in such a way that it is now difficult to imagine our world without them. This, of course, has also impacted the field of criminology, where for decades, genetic identification has been used as a fast and relatively inexpensive method to identify criminals and uncover their actions [1] .

As is known, the main advantages of genetic fingerprinting lie in its high accuracy, allowing for the identification of individuals even with small amounts of biological material. Considering that the analysis is conducted in accordance with all requirements, including repeated experiments, the reliability of such investigations exceeds 99%. This approach has become one of the most important in criminal investigations [2].

In addition to identifying individuals, DNA fingerprinting is also used in many other aspects of forensic research. These include the identification of animal species, plants, and even microorganisms.

At the same time, legislation must be harmonized to implement a comprehensive set of measures in improving livestock breeding, strengthening the feed base, supporting the activities of livestock farms in breeding directions, and promoting large-scale business entities in livestock product production. Accordingly, significant work is being done in our country to increase the total number of livestock, enhance productivity, effectively protect animal health, and ensure epidemiological safety and food product security [3,4].

Although animal DNA identification research is similar to human biological material DNA studies, the process of extracting DNA from biological materials, conducting quantitative and qualitative analysis, and especially interpreting the obtained results differs significantly. Moreover, the distinct procedures for working with the biological materials of each animal necessitate a deeper study of these processes [5].

For studying each pedigree animal, at least three generations of related pedigree data are required. Genetic diseases are identified by mutations in the genes responsible for the development of specific traits. The analysis is carried out on genomic loci responsible for the development of economically significant traits in the livestock allele pool, and based on this, a breeding nucleus is formed—a group of cattle with high productivity potential and resistance to the negative effects of mutations [6]. This task is a serious responsibility for breeding farms, and DNA marking methods are used in one of the stages of solving this task. The results of DNA analysis are crucial for selecting animals with the desired genotypes, as well as for raising and breeding young animals to form a high-performing, genetically uniform, healthy pedigree livestock [7,8].

Materials and methods

Materials

Genetic material (DNA) related to cattle is extracted from biological samples provided for research. These samples may include meat samples, skin samples, hair, horns, and bone samples.

Specific requirements are set for pieces of animals' muscle or other tissues. Their safety can be ensured through the following methods:

- Samples should be cooled or frozen;
- If long-term transport is necessary, especially during hot seasons, samples should be dried in a thin layer on clean paper at room temperature or sprayed with food salt.

Liquid blood samples are dried on a gauze swab. Adding food salt to liquid blood samples is not acceptable.

Methods

Sample treatment:

Small samples were taken from the saliva of 50 cattle gathered from various regions of Uzbekistan, placed in separate sterile test tubes, and recorded with special labels.

DNA extraction:

Method 1: Using the "SilicSorbNA" Reagent for DNA/RNA Extraction ("Rossa" Company)

In this method, depending on the number of objects being tested, 400 µL of "Sorbing Solution" and 12 µL of "Lysis Solution" were added to each tube designated for each object. Then, 100 µL of the sample was added to each tube, mixed, and incubated at 65°C for 1 hour. After incubation, 30 µL of DNA-sorbent was added to each sample, mixed for 2 minutes, and held on a stand. The samples were then centrifuged at 10,000 rpm for 30 seconds, the upper layer was discarded, and 300 µL of "Sorbing Solution" was added and mixed. This process was repeated twice with 500 µL of "Washing Solution," and the final sediment was dried at 65°C for 1 minute. After the sample dried, 100 µL of "Eluting Solution" was added to each sample, and incubation was carried out at 65°C for 5 minutes. After incubation, the samples were centrifuged at 10,000 rpm for 1 minute, and the upper DNA-containing portion was transferred to a clean tube for genotyping [9].

Method 2: Organic Method

According to this method, for each object, 500 µL of a buffer consisting of Tris-NSI, EDTA, sodium chloride, SDS (11), and 20 µL of Proteinase K enzyme (Sigma, USA) was added to the tubes and incubated at 56°C for 1 hour. Then, the samples were deproteinized 3 times using a 25:24:1 ratio of Phenol-Chloroform-Isomyl alcohol mixture. The supernatant from the tubes was collected, and 2.5 times the volume of 96% ethanol was added, and the mixture was kept at -20°C for 24 hours. The samples were then centrifuged at 15,000 rpm for 30 minutes, washed with 70% ethanol, and dried. The dry sediment in the form of DNA in the tubes was dissolved in 50 µL of dH2O and transferred for genotyping [11].

DNA quantification:

The concentration of extracted DNA was quantified by using the Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA) and the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, USA), following the manufacturer's recommended protocols. For each sample, 2 µl of the extracted DNA was mixed with 198 µl of the Qubit working solution before measurement. The DNA quantities extracted from the samples are shown in Table 1.

Table 1: DNA quantities extracted from the samples

Samples	Quantity (ng/µL)
1-sample	0,56
2-sample	0,61
3-sample	0,11
4-sample	0,86
5-sample	0,01
6-sample	0,01
7-sample	0,59
8-sample	0,72
9-sample	0,54
10-sample	0,47
11-sample	0,41
12-sample	0,40
13-sample	0,38
14-sample	0,41
15-sample	0,56
16-sample	0,33
17-sample	0,71
18-sample	0,43
19-sample	0,61
20-sample	0,70

The DNA concentration obtained using extraction methods is presented as follows. The DNA concentrations obtained from 20 samples using extraction methods are shown in **Table 1**.

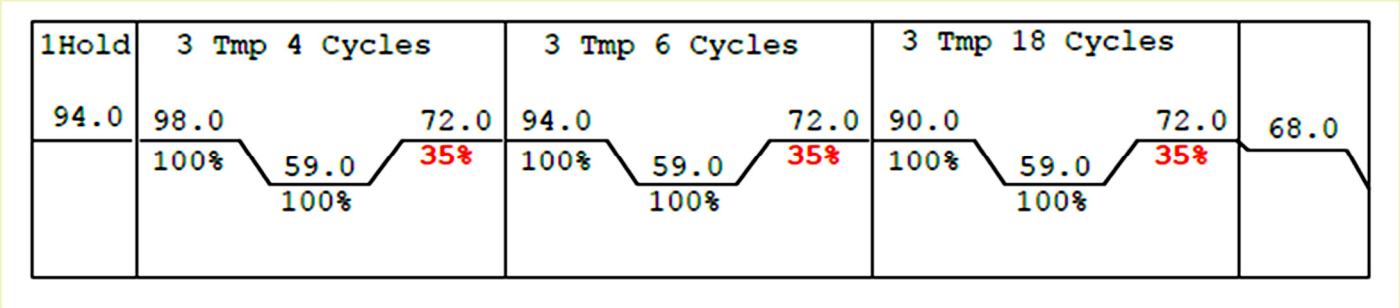
Amplification:

For the PCR amplification process of the extracted cattle DNA samples, the "COrDIS Cattle" kit was used. 5 µL of the Activator solution from the kit was added to each test tube. Then, the total volume of the liquid was adjusted to 20 µL by adding Genomic DNA in the range of 0.2-2 ng. The optimal amount of DNA to be added is 1 ng. The volume of the added DNA depends on its concentration. The maximum possible volume of the DNA solution to be added is 20 µL. If necessary, the total reaction volume can be increased up to 25 µL by adding deionized water included in the kit. After all components have been added, it is recommended to mix the reaction mixture 5-8 times or use a vortex until it reaches a homogeneous state. If necessary, the solution can be collected at the bottom of the test tube by short centrifugation. For optimal reaction efficiency, thorough mixing is essential. For each sample being tested, one positive control (1 µL of control DNA from the kit) and one negative control (deionized water instead of DNA) should be amplified.

The following amplification requirements are recommended as standard parameters (Table 2). It is important to control the heating rate during the temperature rise from 59°C to 72°C to be 0.3°C per second. Due to the high complexity of the amplification involving 15 primer pairs, this heating rate is crucial for the optimal efficiency of the reaction [10].

For obtaining a complete STR profile of the sample, 0.2 nanograms of extracted DNA is sufficient. The optimal amount is 1 nanogram. PCR was performed on the samples using ProFlex PCR System (Applied Biosystems, USA) and the PCR products were analyzed using the SeqStudio 8 Flex genetic analyzer (Hitachi, Japan)

Table 2: Amplification parameters



Results

After the analysis is completed, it is necessary to evaluate the results of analyzing the positive control sample. The genotype of the obtained positive control sample was compared with the genotype specified in the manual. Since the genotype of the positive control sample completely matched the expected sample, the genotyping of the analyzed samples continued (Figure 1 and Figure 2).

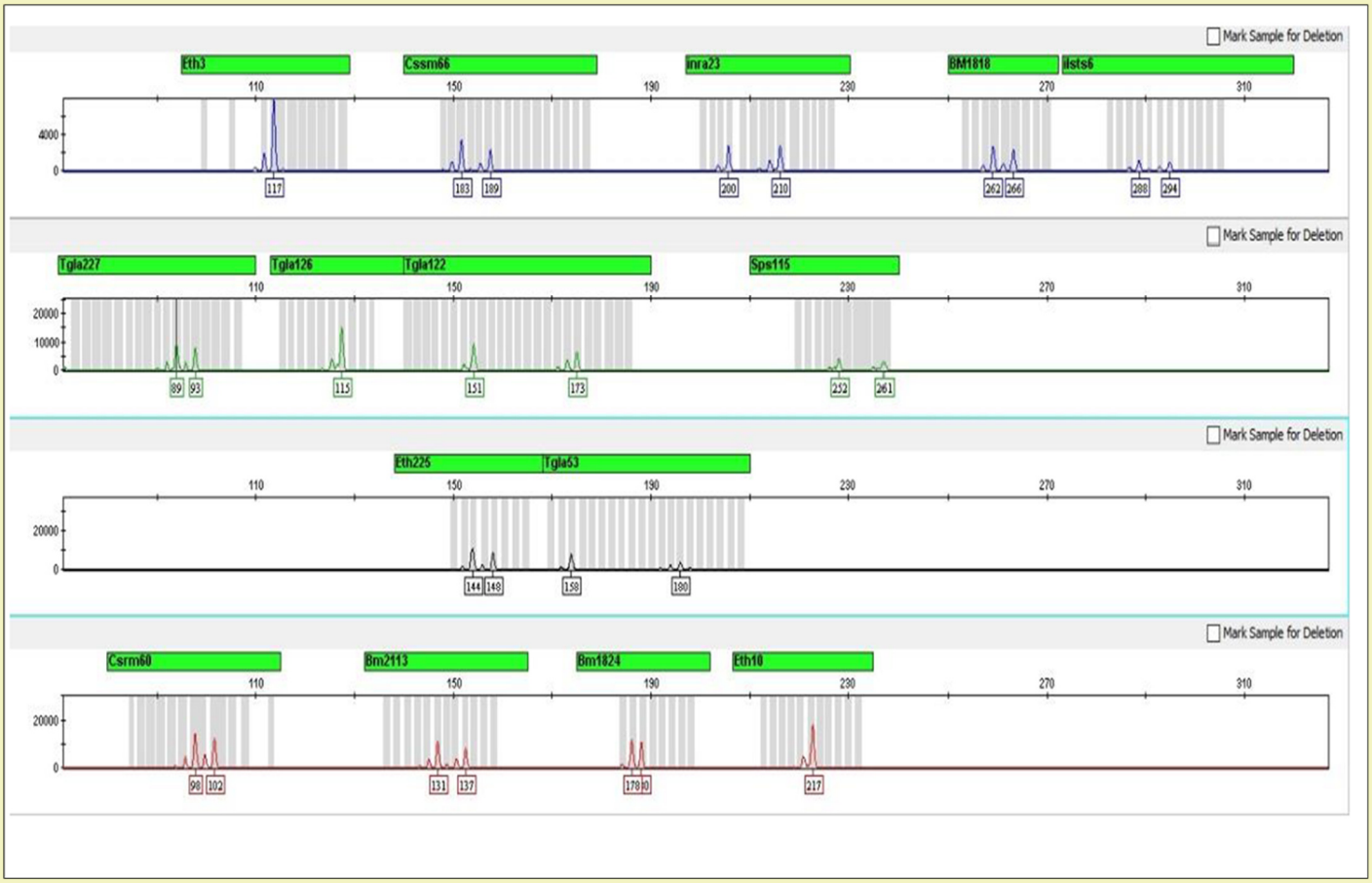


Figure 1: Fragment analysis spectrogram of the animal genome (sample indicator provided in the set)

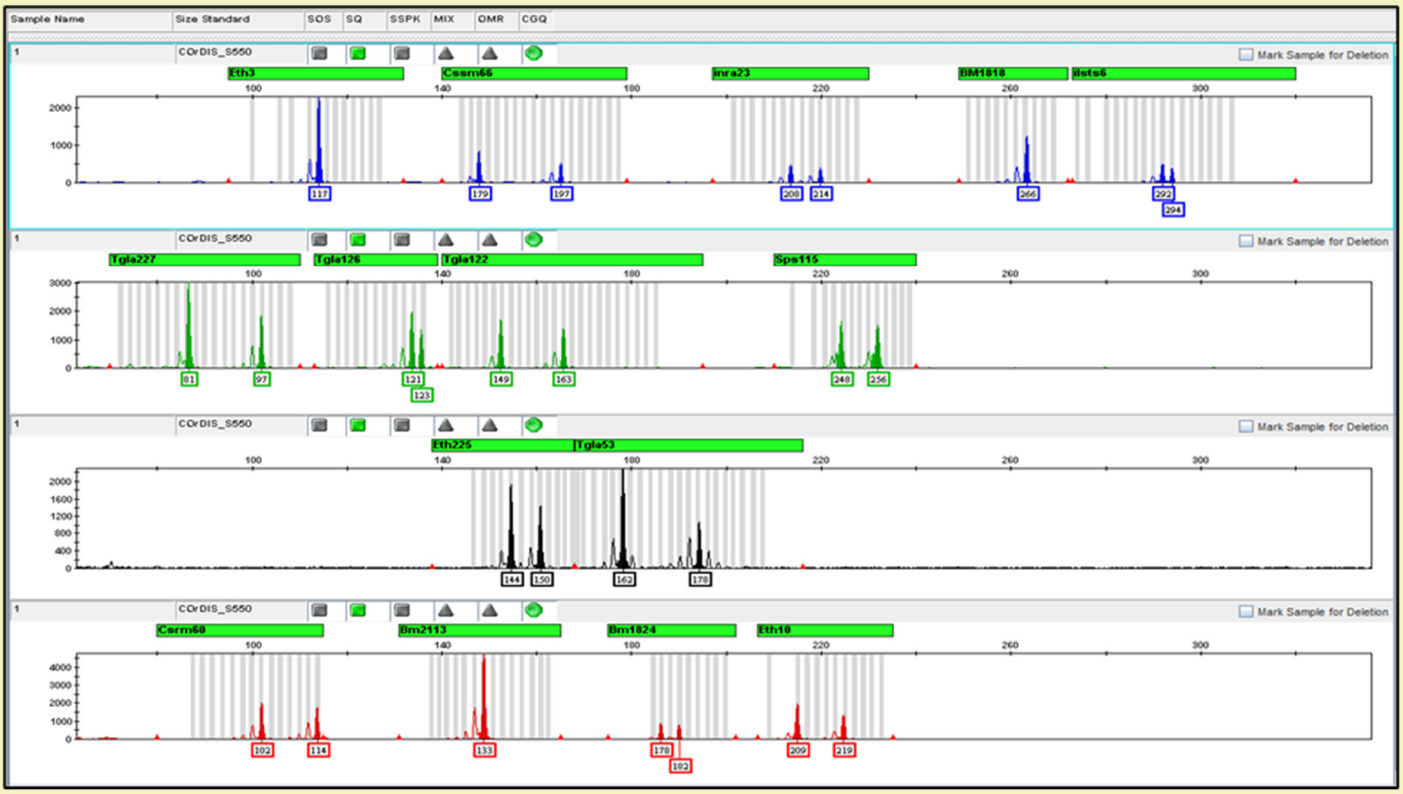


Figure 2: Spectrogram of the cattle genotype (Result obtained at the end of the research)

Each marker locus in the electropherogram may correspond to one or two PCR products, corresponding to homozygous and heterozygous states. The difference in allele length is usually 2 base pairs, reflecting differences in the number of dinucleotide repeats. To correctly determine the genotype, it is necessary to take into account the nature of the stutter. Stutters are the extra products of microsatellite marker amplification. For dinucleotide markers specific to all loci in the Cordis Cattle set, it is typical for the stutter to be -2 base pairs relative to the main product. The intensity of the stutter signal can reach up to 50% of the intensity of the allele product [10]. If the difference in allele length is 2 base pairs, the stutter of the longer allele overlaps with the shorter allele, significantly increasing the signal level [12] (Figure 3).

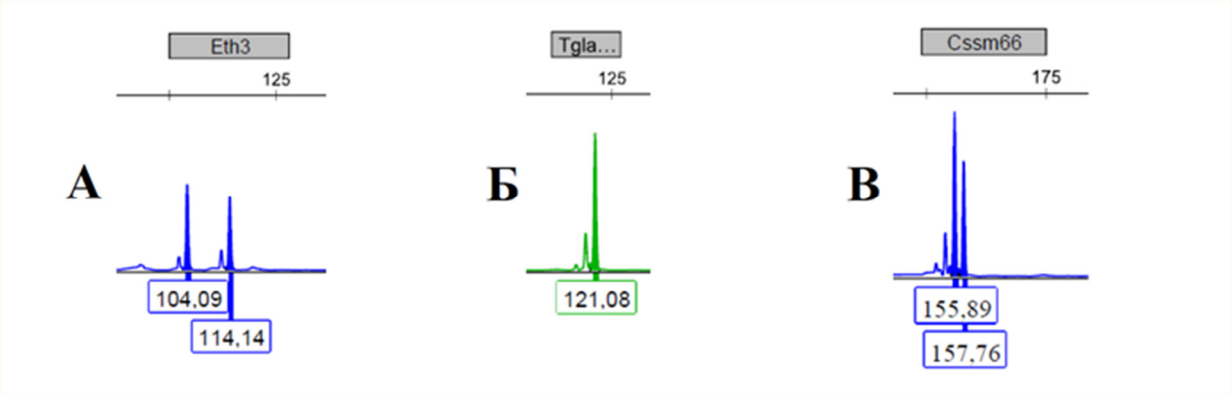


Figure 3:
А– Example of a 104/114 heterozygous genotype at the ETH3 locus,
Б– Example of 121 homozygosity at the TGLA126 locus,
В– Example of a 155/157 heterozygous genotype at the CSSM locus, where the stutter corresponds to the 155 allele.

Conclusion

The results of the conducted research can be effectively used in genetic expertise, as well as in fundamental and applied studies aimed at examining the allele pool status and dynamics of animal populations, which are agricultural objects. It can also contribute to the development of methods for managing the gene pool of breeds. Through the identification of animal biomaterials, it will be possible to effectively organize the breeding of economically valuable animals, increase their productivity, and protect their health. Additionally, these studies can be applied in the future to other animals, such as identifying the meat, skin, and other biological materials of stolen horses or animals listed in the “Red Book” when they are hunted, thus implementing extensive measures for their identification.

References:

1. *https://biomolecula.ru/articles/kriminalistika-molekuliarno-geneticheskaia-ekspertiza.*
2. *The evaluation of forensic DNA evidence. Washington (DC): National Academies Press, 1996.*
3. *Shang-Yin Vanson Liu, Chia-Ling Carynn Chan, Oceana Lin, Chieh-Shen Hu, Chaolun Allen Chen. (2013). DNA Barcoding of Shark Meats Identify Species Composition and CITES-Listed Species from the Markets in Taiwan. PLoS ONE. 8, e79373.*
4. *Julien Bénard-Capelle, Victoire Guillonneau, Claire Nouvian, Nicolas Fournier, Karine Le Loët, Agnès Dettai. (2015). Fish mislabelling in France: substitution rates and retail types. PeerJ. 2, e714.*
5. *Georges M., Massey J.M. Velogenetics, or the synergistic use of marker assisted selection and germ line manipulation // Theriogenology. 1991.-V. 35.-P. 151-159.*
6. *Haley C.S., Visscher P.M. Strategies to utilize marker - quantitative trait loci associations // J. Dair. Sci. 1998.-V. 81, № 2.-C. 85-97.*
7. *Meuwissen T.H.E.. Genomic selection: The future of animal breeding // Norwegian University of LifSciences, Box 5003, 1432 As Norway.2007–P. 88-91.*
8. *Sax K. The association of size differences with seed-coat pattern and pigmentation in Phaseolus Vulgaris //Genetics.-1923.-V. 8.-P.552-560.*
9. *Instruction for use of the SilicSorbNA kit.*
10. *Instruction for use of the COrDIS Cattle kit.*
11. *S.A. Ataxodjayev and others “Forensic DNA Expertise of Humans.” Educational manual, 2011.*
12. *Saitova N.S. “The Importance of Animal DNA in Criminal Investigation,” Tashkent, Seminar Proceedings titled “Current Issues in Forensic Biology Expertise,” April 25-26, 2024.*

Tiny Partitions, Big Impact: Forensic Applications of Digital PCR – A Review

Ms. Rita Fakhr[1], Ms. Un Na Koh[1], Ms. So Eun Lee[1], Prof Si-Keun Lim[1,2,3]*
[1] Department of Forensic Sciences, Sungkyunkwan University, Suwon, Republic of Korea
[2] ID-Cell Forensics Co., Sungkyunkwan University, Suwon, Republic of Korea
[3] Convergence Bio Forensic Institute (CBFI), Biomedical Institute for Convergence at Sungkyunkwan University (BICS), Sungkyunkwan University, Suwon, Republic of Korea
**Email: sikeun.lim@skku.edu*

Abstract

Digital PCR (dPCR) emerged in the late 1990s as an alternative to quantitative PCR (qPCR) for absolute nucleic acid quantification. By partitioning samples into thousands of individual reactions, dPCR enhances sensitivity and enables precise detection of low-abundance targets without the need for standard curves. These advantages make it particularly valuable in forensic and biomedical sciences. The commercialization of droplet-based and chip-based dPCR platforms has further expanded its accessibility and usability. Our laboratory is among the few applying droplet digital PCR (ddPCR) in forensic research, contributing to its advancement in the field. As dPCR adoption continues to grow, comprehensive validation and standardization will be essential for its integration into routine practice.

Keywords: biomedical, dPCR, forensic science.

Introduction

The concept of digital PCR (dPCR) was first introduced in the late 1990s as an alternative approach to quantitative PCR (qPCR), offering a method for absolute quantification of nucleic acids. Early iterations of dPCR, were “single-molecule PCR” or “limiting dilution PCR” [1,2]. Vogelstein and Kinzler (1999) pioneered the dPCR technique, demonstrating its utility in detecting rare genetic mutations with increased sensitivity. The early implementations of dPCR involved limiting dilution methods, where samples were distributed into individual reaction wells, each containing either a single target molecule or none [1]. This foundational work laid the groundwork for modern dPCR platforms, which have since evolved to include microfluidic and droplet-based partitioning systems. dPCR has revolutionized nucleic acid quantification, offering absolute quantification capabilities with high sensitivity and precision. Unlike qPCR, which relies on cycle threshold (Ct) values and fluorescence intensity, dPCR partitions the sample into thousands of individual reactions, enabling absolute quantification without the need for standard curves [3]. This approach eliminates variability caused by preferential amplification, making dPCR especially valuable for detecting low-abundance targets and rare mutations. As a result, it has become a powerful tool in diverse scientific and medical applications (Figure 1) [4,5]. In forensic science, dPCR has proven to be particularly valuable for analyzing trace DNA in complex mixtures, enhancing the accuracy of short tandem repeat (STR) profiling [6,7]. Additionally, it facilitates forensic age prediction via DNA methylation markers [8–11], and enables

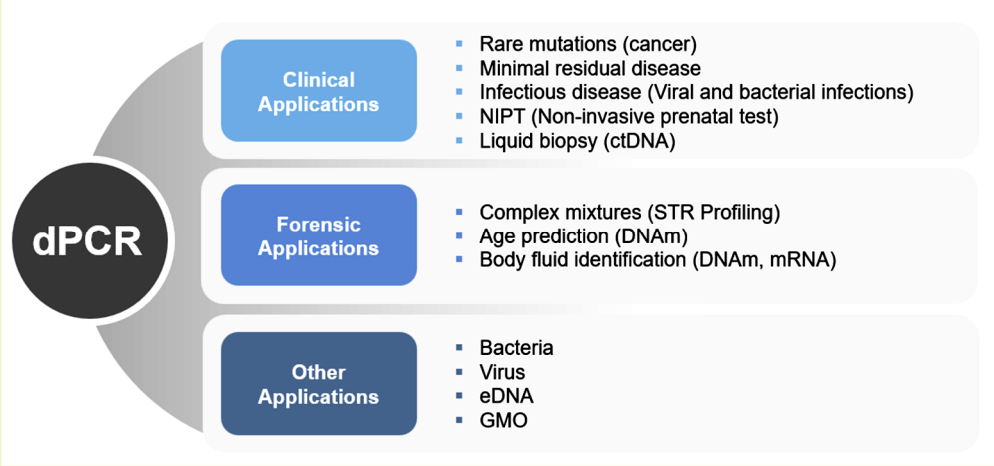


Figure 1: Fields of application of dPCR.

body fluid identification through RNA [12] and methylation-based assays [13]. In clinical diagnostics, dPCR has become indispensable for detecting low-frequency cancer mutations in cancer [14], monitoring minimal residual disease [15,16], and quantifying viral loads in infectious diseases [17]. Its applications extend to non-invasive prenatal testing (NIPT) [18] and liquid biopsy analyses, where it enables sensitive detection of rare circulating tumor DNA (ctDNA) [19,20]. Furthermore, dPCR is extensively used in pathogen detection and microbiology due to its exceptional tolerance to PCR inhibitors, therefore allowing highly precise quantification of viral pathogens in wastewater surveillance [21,22]. The technology has also gained prominence in environmental DNA (eDNA) studies [23–26] providing sensitive detection of genetic material across ecosystems, which is crucial for biodiversity assessment, monitoring invasive species, and studying microbial populations. In agricultural and food safety applications, dPCR offers robust solutions for genetically modified organism (GMO) detection and quantification [27–29].

The commercialization of dPCR technology in the early 2010s marked a significant turning point, with major biotechnology companies developing high-throughput and user-friendly platforms. Bio-Rad introduced the first droplet digital PCR (ddPCR) system (Figure 2A) which uses water-oil emulsion droplets to divide DNA samples into thousands of individual nanoliter reactions. Other manufacturers, including Thermo Fisher Scientific, QIAGEN, Roche Diagnostics (Figure 2B), and Stilla Technologies, have also developed alternative dPCR platforms using chip-based and crystal droplet technologies, further expanding the capabilities of this approach.

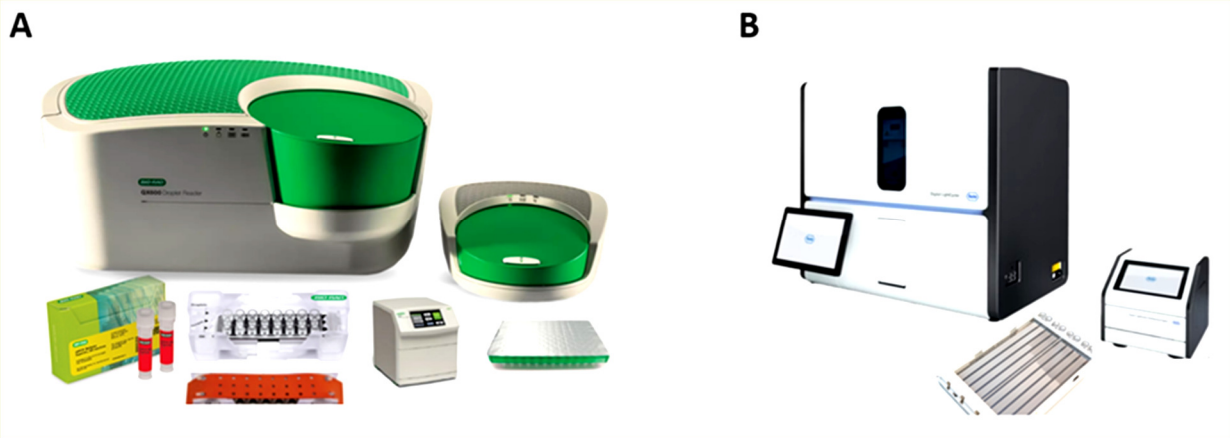


Figure 2: ddPCR and dPCR instruments.
A) QX600™ Droplet Digital PCR™ System with all its consumables, by Bio-Rad, USA.
QX600 offers 6-color multiplexing (FAM, HEX, Cy5, Cy5.5, ROX, ATTO590), allowing a clear discrimination between multiple targets.
B) Digital LightCycler® System with all its consumables, by Roche Diagnostics, Switzerland.
Digital LightCycler offers 6-color multiplexing (FAM, HEX, Cy5, Cy5.5, Texas Red, Coumarin 6), allowing a clear discrimination between multiple targets.
www.bio-rad.com/en
www.roche.com

This mini-review paper focuses on dPCR, outlining its workflow, key experimental considerations, and advantages over qPCR while also highlighting its emerging applications in forensic and clinical research, including assay development efforts from our laboratory.

dPCR Workflow and Experimental Design

dPCR, (including ddPCR) workflow typically follows five key steps: (1) sample preparation, (2) partitioning, (3) PCR amplification, (4) signal detection, and (5) data analysis (Figure 3).

Sample Preparation and Partitioning

The reaction mixture consists of the nucleic acids (DNA/RNA), target-specific primers, fluorescent probes, and a specialized master mix. In ddPCR systems, the sample is emulsified into thousands of nanoliter-sized droplets using a droplet generator. Chip-based dPCR platforms instead use microfluidic arrays to create physically isolated partitions [3]. Each partition contains either zero, one, or a few copies of the target nucleic acid, enabling single-molecule detection [1].

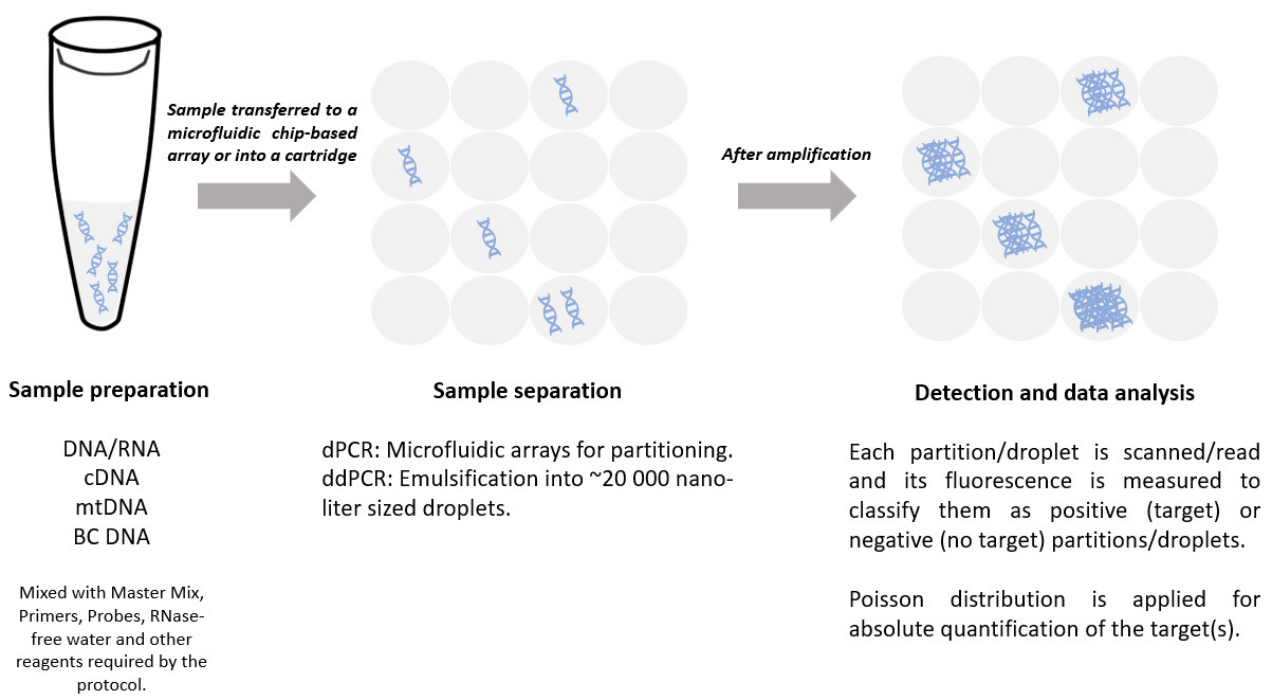


Figure 3: Experimental principle of dPCR/ddPCR.
Sample is prepared according to the manufacturer's protocol and adjustment can be made for assay optimization. A specific volume of the mixture is then transferred into the microfluidic chip-based array (dPCR) or into a cartridge (ddPCR) for sample partitioning. Amplification then takes place in each partition. Subsequently, each partition is scanned/read for target absolute quantification.

PCR Amplification and Signal Detection

Following partitioning, thermal cycling amplifies the target sequences independently within each partition. Unlike qPCR – where all templates compete in bulk reaction – dPCR's compartmentalization eliminates template competition. This isolation:

- Enhances sensitivity for rare allele detection [30]
- Reduce bias from PCR inhibitors [5]
- Enables absolute quantification of low-abundance targets [5]

Fluorescent probes allow binary classification of partitions as positive (target detected) or negative (no target).

Data Analysis

A droplet reader (ddPCR) or microfluidic scanner (chip-based dPCR) measures the fluorescence intensity in each partition. Using Poisson statistics, the absolute target concentration is calculated from the ratio of positive to negative partitions, eliminating the need for a standard curve [3].

Comparison of dPCR and qPCR

qPCR remains the gold standard for nucleic acid quantification due to its widespread adoption and extensive validation across various applications. qPCR measures fluorescence during each amplification cycle, allowing relative quantification via cycle threshold (Ct) values compared to a standard curve. In contrast, dPCR employs endpoint absolute quantification, directly counting target molecules without relying on amplification efficiency or reference standards [3].

Key Advantages of dPCR

- Partitioning strategy: By dividing reactions into thousands of nanoscale partitions, dPCR reduces template competition, enhancing sensitivity for detection of:
  - Low-abundance targets
  - Rare mutations within a background of wild-type sequences or highly abundant DNA [31].
- Reduced bias: Eliminate variability from amplification efficiency differences or standard curve inaccuracies.
- Downstream compatibility (ddPCR-specific): ddPCR allows post-amplification DNA recovery for additional analyses [6,7].

Limitations of dPCR

- Contamination risks: Sample transfer steps increase susceptibility to cross-contamination [3].
- Higher costs: Reagents and instrumentation are more expensive than qPCR, limiting routine use.

While qPCR continues to be the preferred method for DNA quantification due to its established workflows and cost-effectiveness, further rigorous validation and standardization of dPCR protocol is needed to expand its use in routine molecular diagnostics.

Applications of ddPCR in Forensic and Biomedical Sciences

1. Forensic Genetics

Mixture Analysis for STR Profiling

Short tandem repeat (STR) analysis is the gold standard for forensic DNA profiling, enabling individual identification and case resolution [32]. While traditional PCR and qPCR methods dominate forensic workflows, they struggle with mixed DNA samples, where distinguishing allelic variants from minor contributors in mixed DNA samples can be difficult. dPCR, particularly ddPCR, offers a significant advantage in these situations by partitioning DNA into thousands of individual droplets, allowing for more precise detection of minor contributors within a mixture. This capability is especially valuable in forensic investigations involving complex biological samples, such as sexual assault cases, where a low-level DNA contributor might otherwise be masked by a predominant profile. Based on previous research [6,7], our laboratory has optimized post-ddPCR DNA recovery from ddPCR droplets, optimizing the process to enhance sensitivity. By modifying the extraction methodology, the efficiency of STR analysis in forensic casework has been improved, enabling a more accurate interpretation of DNA mixtures.

Epigenetics

DNA methylation at age-associated CpG sites exhibits a well-documented correlation with chronological age, enabling forensic age estimation [33,34]. ddPCR-based methylation assays have been designed to assess age-associated CpG sites, providing a precise and reliable approach for forensic age estimation [8–11]. Beyond age prediction, tissue-specific DNA methylation patterns permit the identification of biological fluids, such as blood, saliva, and semen, enhancing forensic casework by aiding in crime scene reconstruction [13]. Our laboratory has developed a hexaplex ddPCR assay (Figure 4) targeting three age-associated methylation markers for forensic age

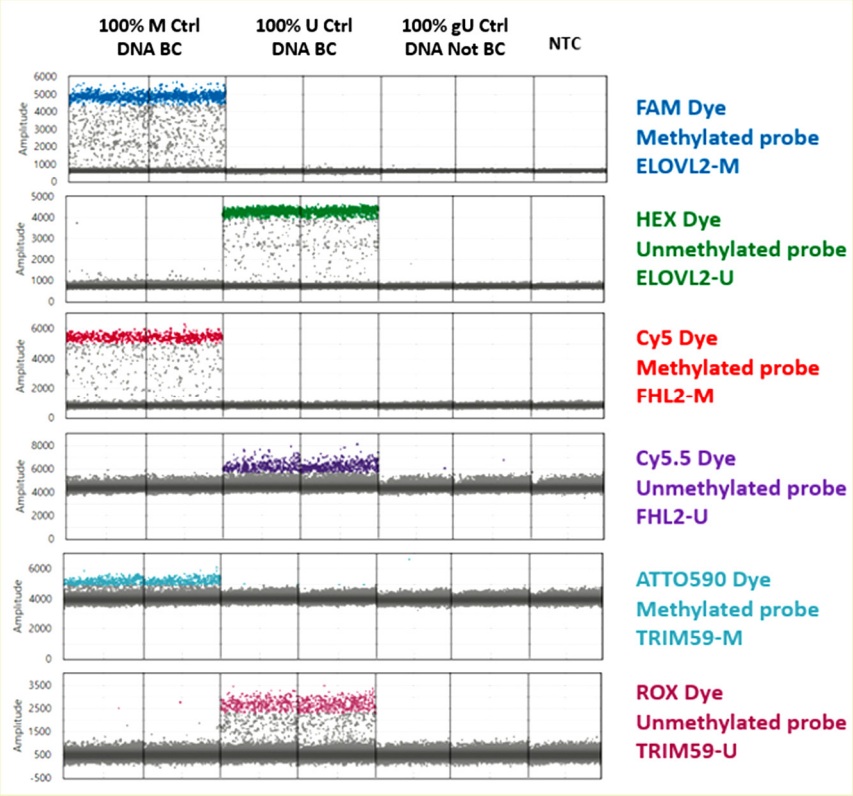


Figure 4: 1-D amplitude plot of a ddPCR result.
This plot comes from an ongoing age prediction study using three age-related markers: ELOVL2, FHL2, and TRIM59.
For each marker, a methylated and unmethylated probe were designed to distinguish the methylation status of the targeted CpG sites.
Shown here are specificity results for all probes. 100% methylated and unmethylated bisulfite converted DNA and 100% unmethylated non-bisulfite conversion DNA were used for the specificity experiment.
The colored dots represent the positive droplets, and the grey dots represent the baseline (negative droplets).
All probes exhibited high specificity to their specific target.

prediction and is currently undergoing validation. Concurrently, we are also developing a multiplex methylation assay to simultaneously identify semen and saliva in a single reaction. This dual-target approach improves the resolution of body fluid mixtures and minimizes the likelihood of false positives, ultimately optimizing forensic DNA analysis.

RNA Analysis for Body Fluid Identification

The use of RNA markers for forensic body fluid identification has gained attention, as RNA expression patterns differ between bodily secretions. These differences allow forensic scientists to distinguish among biological materials such as saliva, semen, blood and menstrual blood [35]. dPCR has been used to detect RNA biomarkers for these body fluids, particularly in cases where DNA is degraded or absent. Our laboratory has recently developed a two-step reverse transcription ddPCR (RT-ddPCR) assay that simultaneously identify saliva and semen [12]. This multiplex assay enhances forensic body fluid analysis by improving the detection accuracy of RNA biomarkers, thereby reducing the risk of misclassification in mixed or degraded samples. The integration of ddPCR in forensic RNA analysis represents a significant advancement, offering a robust and reliable method for body fluid identification in forensic investigations.

Microbiome Analysis

The human microbiome, including bacteria present in different body sites, can serve as an additional forensic tool. Forensic microbiome analysis has gained popularity for its potential in postmortem interval estimation, and geolocation, as microbial communities are informative and can change predictably after death [36]. Mitochondrial DNA (mtDNA) analysis complements microbiome investigations through its high copy number in degraded or limited forensic samples, where nuclear DNA may be insufficient [37]. Our laboratory employs ddPCR technology to simultaneously quantify both bacterial biomarkers and human mtDNA. The method's high sensitivity and absolute quantification capability allows accurate measurement of microbial DNA and mtDNA even in degraded or low-template samples. This dual-target approach enhances forensic casework by providing additional layer of information, whether for human identification or microbiome analysis.

2. Biomedical Sciences

In oncology, dPCR is widely used for detecting low-abundance mutations, assessing gene expression levels, and monitoring minimal residual disease in patients undergoing treatment. Its ability to precisely quantify rare genetic variants makes it a valuable tool for early cancer detection and advancement of personalized medicine. Similarly, this technology plays a crucial role to quantify low viral and bacterial loads with high accuracy in infectious disease diagnostics [38–40], which is especially important in clinical diagnostics, epidemiology, and blood screening. Our lab applies ddPCR technology to ensure the integrity of reference materials before they are used. Specifically, we are currently developing a pentaplex ddPCR assay to simultaneously screen for HIV-1, HIV-2, HCV, and HBV in blood samples intended for use as reference materials. The assay also includes an internal control to verify assay performance and minimize the risks of false negative results. This step is critical to confirm that the blood samples are free of viral contamination before they are distributed to experimenters for downstream analyses.

Conclusion

Digital PCR's ability to deliver absolute quantification, enhanced sensitivity, and greater resistance to inhibitors, makes it a strong alternative to qPCR across many applications. While its forensic applications are still emerging, dPCR or ddPCR, has shown promise in STR mixture analysis, age estimation, body fluid identification, and microbiome analysis. Our laboratory is among the few pioneering the use of this technology in forensic research via developing ddPCR assays aimed at broadening its forensic utility. As ddPCR adoption continue to grow, rigorous validation and standardization will be essential for integration this powerful tool into routine forensic casework.

References:

1. *Vogelstein B, Kinzler KW. Digital PCR [Internet]. Vol. 96, Genetics. 1999. Available from: www.pnas.org.*
2. *Morley AA. Digital PCR: A brief history. Vol. 1, Biomolecular Detection and Quantification. Elsevier GmbH; 2014. p. 1–2.*
3. *Quan PL, Sauzade M, Brouzes E. DPCR: A technology review. Vol. 18, Sensors (Switzerland). MDPI AG; 2018.*
4. *Sreejith KR, Ooi CH, Jin J, Dao DV, Nguyen NT. Digital polymerase chain reaction technology-recent advances and future perspectives. Vol. 18, Lab on a Chip. Royal Society of Chemistry; 2018. p. 3717–32.*
5. *Mirabile A, Sangiorgio G, Bonacci PG, Bivona D, Nicitra E, Bonomo C, et al. Advancing Pathogen Identification: The Role of Digital PCR in Enhancing Diagnostic Power in Different Settings. Vol. 14, Diagnostics. Multidisciplinary Digital Publishing Institute (MDPI); 2024.*
6. *Dutra L, Franz O, Puupponen VM, Tiirola M. DNA recovery from Droplet Digital™ PCR emulsions using liquid nitrogen. Biotechniques. 2020 Dec 1;69(6):451–4.*
7. *Zhu Q, Wang H, Cao Y, Huang Y, Hu Y, Zhou Y, et al. Evaluation of microfluidics-based droplet PCR combined with multiplex STR system in forensic science. Electrophoresis. 2022 Apr 1;43(7–8):848–56.*
8. *Ho Lee M, Hee Hwang J, Min Seong K, Jin Ahn J, Jun Kim S, Yong Hwang S, et al. Application of droplet digital PCR method for DNA methylation-based age prediction from saliva. Leg Med. 2022 Feb 1;54.*
9. *Manco L, Dias HC. DNA methylation analysis of ELOVL2 gene using droplet digital PCR for age estimation purposes. Forensic Sci Int. 2022 Apr 1;333.*
10. *Dias HC, Manco L. Predicting age from blood by droplet digital PCR using a set of three DNA methylation markers. Forensic Sci Int. 2024 Mar 1;356.*
11. *Zhou Y, Wang Y, Song M, Jiang L, Sun C, Wang S, et al. A high-throughput droplet digital PCR system aiming eight DNA methylation targets for age prediction. J Pharm Biomed Anal. 2024 Mar 15;240.*
12. *Lee SE, Koh UN, Fakhr R, Lim SK. Development of two step reverse transcription droplet digital PCR (RT-ddPCR) for simultaneous identification of saliva and semen. Leg Med. 2024 Nov 1;71.*
13. *Kader F, Ghai M, Olaniran AO. Characterization of DNA methylation-based markers for human body fluid identification in forensics: a critical review. Vol. 134, International Journal of Legal Medicine. Springer; 2020.*
14. *Zhao Y. A comparative study of ddPCR and sanger sequencing for quantitative detection of low-frequency mutation rate. In: IOP Conference Series: Earth and Environmental Science. Institute of Physics Publishing; 2019.*
15. *Bernardi S, Malagola M, Farina M, Polverelli N, Re F, Russo D. Digital PCR as a New Method for Minimal Residual Disease Monitoring and Treatment Free Remission Management in Chronic Myeloid Leukemia Patients: Is It Reliable? Vol. 4, Hemato. Multidisciplinary Digital Publishing Institute (MDPI); 2023. p. 1–11.*
16. *Mao S, Lin Y, Qin X, Miao Y, Luo C, Luo C, et al. Droplet digital PCR: An effective method for monitoring and prognostic evaluation of minimal residual disease in JMML. Br J Haematol. 2024 Jun 1;204(6):2332–41.*
17. *Chen B, Jiang Y, Cao X, Liu C, Zhang N, Shi D. Droplet digital PCR as an emerging tool in detecting pathogens nucleic acids in infectious diseases. Vol. 517, Clinica Chimica Acta. Elsevier B.V.; 2021. p. 156–61.*
18. *Guo Y, Charoenkwan P, Traisrisilp K, Piyamongkol W, Tongprasert F. Application of Digital Polymerase Chain Reaction (dPCR) in Non-Invasive Prenatal Testing (NIPT). Biomolecules [Internet]. 2025 Mar 1;15(3):360. Available from: https://www.mdpi.com/2218-273X/15/3/360.*
19. *Jahani MM, Mashayekhi P, Omrani MD, Meibody AA. Efficacy of liquid biopsy for genetic mutations determination in non-small cell lung cancer: a systematic review on literatures. BMC Cancer [Internet]. 2025 Mar 11;25(1):433. Available from: https://bmccancer.biomedcentral.com/articles/10.1186/s12885-025-13786-w*
20. *Gassa A, Fassunke J, Schueten S, Kuhlmann L, Scherer M, Qien J, et al. Detection of circulating tumor DNA by digital droplet PCR in resectable lung cancer as a predictive tool for recurrence. Lung Cancer. 2021 Jan 1;151:91–6.*
21. *Jahne MA, Brinkman NE, Keely SP, Zimmerman BD, Wheaton EA, Garland JL. Droplet digital PCR quantification of norovirus and adenovirus in decentralized wastewater and graywater collections: Implications for onsite reuse. Water Res. 2020 Feb 1;169.*
22. *Kiulia NM, Gonzalez R, Thompson H, Aw TG, Rose JB. Quantification and Trends of Rotavirus and Enterovirus in Untreated Sewage Using Reverse Transcription Droplet Digital PCR. Food Environ Virol. 2021 Jun 1;13(2):154–69.*
23. *Kokkoris V, Vukicevich E, Richards A, Thomsen C, Hart MM. Challenges Using Droplet Digital PCR for Environmental Samples. Appl Microbiol. 2021 May 21;1(1):74–88.*
24. *Klymus KE, Merkes CM, Allison MJ, Goldberg CS, Helbing CC, Hunter ME, et al. Reporting the limits of detection and quantification for environmental DNA assays. Environmental DNA. 2020 Jul 1;2(3):271–82.*
25. *Hunter ME, Dorazio RM, Butterfield JSS, Meigs-Friend G, Nico LG, Ferrante JA. Detection limits of quantitative and digital PCR assays and their influence in presence–absence surveys of environmental DNA. Mol Ecol Resour. 2017 Mar 1;17 (2):221–9.*
26. *Baker CS, Steel D, Nieukirk S, Klinck H. Environmental DNA (eDNA) from the wake of the whales: Droplet digital PCR for detection and species identification. Front Mar Sci. 2018 Apr 19;5(APR).*
27. *Bogožalec Košir A, Demšar T, Štebih D, Žel J, Milavec M. Digital PCR as an effective tool for GMO quantification in complex matrices. Food Chem. 2019 Oct 1;294:73–8.*
28. *Köppel R, Bucher T. Rapid establishment of droplet digital PCR for quantitative GMO analysis. European Food Research and Technology. 2015 Sep 8;241(3):427–39.*

References (cont'd):

29. *Dobnik D, Štebih D, Blejec A, Morisset D, Žel J. Multiplex quantification of four DNA targets in one reaction with Bio-Rad droplet digital PCR system for GMO detection. Sci Rep. 2016 Oct 14;6.*
30. *Vynck M, Chen Y, Gleerup D, Vandesompele J, Trypsteen W, Lievens A, et al. Digital PCR Partition Classification. Vol. 69, Clinical Chemistry. Oxford University Press; 2023. p. 976–90.*
31. *Whale AS, Cowen S, Foy CA, Huggett JF. Methods for Applying Accurate Digital PCR Analysis on Low Copy DNA Samples. PLoS One. 2013 Mar 5;8(3).*
32. *Linacre A, Templeton JEL. Forensic DNA profiling: state of the art. Research and Reports in Forensic Medical Science. 2014 Aug;25.*
33. *Cho S, Jung SE, Hong SR, Lee EH, Lee JH, Lee SD, et al. Independent validation of DNA-based approaches for age prediction in blood. Forensic Sci Int Genet. 2017 Jul 1;29:250–6.*
34. *Zbieć-Piekarska R, Spólnicka M, Kupiec T, Parys-Proszek A, Makowska Z, Pałeczka A, et al. Development of a forensically useful age prediction method based on DNA methylation analysis. Forensic Sci Int Genet. 2015 Aug 7;17:173–9.*
35. *Nussbaumer C, Gharehbaghi-Schnell E, Korschineck I. Messenger RNA profiling: A novel method for body fluid identification by Real-Time PCR. Forensic Sci Int. 2006 Mar 10;157(2–3):181–6.*
36. *Metcalf JL, Xu ZZ, Bouslimani A, Dorrestein P, Carter DO, Knight R. Microbiome Tools for Forensic Science. Vol. 35, Trends in Biotechnology. Elsevier Ltd; 2017. p. 814–23.*
37. *Court DS. Mitochondrial dna in forensic use. Vol. 5, Emerging Topics in Life Sciences. Portland Press Ltd; 2021. p. 415–26.*
38. *Kojabad AA, Farzanehpour M, Galeh HEG, Dorostkar R, Jafarpour A, Bolandian M, et al. Droplet digital PCR of viral DNA/RNA, current progress, challenges, and future perspectives. Vol. 93, Journal of Medical Virology. John Wiley and Sons Inc; 2021. p. 4182–97.*
39. *Ciesielski M, Blackwood D, Clerkin T, Gonzalez R, Thompson H, Larson A, et al. Assessing sensitivity and reproducibility of RT-ddPCR and RT-qPCR for the quantification of SARS-CoV-2 in wastewater. J Virol Methods. 2021 Nov 1;297.*
40. *Li M, Lv Y, Cui D, Xu Y, Lin M, Zhang X, et al. Development and clinical validation of a one-step pentaplex real-time reverse transcription PCR assay for detection of hepatitis virus B, C, E, Treponema pallidum, and a human housekeeping gene. BMC Infect Dis. 2023 Dec 1;23(1).*

Technical Article

Construction and Application of an Intelligent Fully Automated Physicochemical Analytical Laboratory

Mr. Tie Nan, Mrs. Zhao Jingyou, Mr. Zhou Ruhua, Mr. Gong Duo, Mr. Li Min
Institute of Criminal Science and Technology of Suzhou Public Security Bureau, People's Republic of China

Abstract

Objective: To establish an integrated physicochemical fully automated robotic laboratory which incorporates preprocessing, sample loading, and result analysis.

Methods: A unified preprocessing platform was designed by integrating extraction, centrifugation, and concentration procedures. A robotic arm with capabilities such as grasping, moving, cap twisting, and quantitative liquid dispensing was developed, coupled with a liquid-level image recognition system. Mobile coordination among hybrid robots enabled signal interaction between the preprocessing platform and analytical instruments. Spectrum interpretation software was engineered to automate the screening of narcotics and toxic substances.

Results: A fully automated intelligent physicochemical laboratory was successfully constructed.

Conclusion: The implementation of this automated robotic laboratory has achieved high efficiency and unmanned testing, marking a breakthrough in the automation of forensic technology applications.

Keywords: Physicochemical testing, Automation, Forensic technology

Introduction

Physicochemical testing, as an important branch of forensic science, plays a crucial role in judicial identification and other fields as the public security and social security system continues to improve. The physical and chemical testing process typically involves sample pretreatment and instrumental analysis. Although some automation has been achieved in the sample pretreatment and instrumental analysis stages, certain steps, such as, adding extraction solvents, mixing, centrifuging, sample transfer, and evaporation to a constant volume still require manual intervention. Moreover, chromatogram analysis and outcome assessment are entirely dependent on the testing personnel. Faced with the current massive volume of samples, the high-intensity and repetitive multi-step operations not only increase the risk of human error and cross-contamination, but also lead to reduced testing efficiency and accuracy, as well as the inability to trace the results. Additionally, toxic and harmful chemical reagents pose significant risks to the health of the testing personnel. Therefore, the establishment of a fully automated high-throughput physical and chemical testing laboratory that integrates sample pretreatment, automatic sample loading, and result screening will have broad application value in the automated screening of drugs and poisons.

Composite robot, which combines the flexible mobility of a mobile platform with the precise operation capability of a robotic arm, can autonomously perform a series of tasks, such as, material handling, assembly, and quality inspection [1]. Therefore, this laboratory has developed a fully automated physical and chemical pretreatment platform using collaborative robotic arms and introducing composite robots to build an intelligent fully automated physical and chemical testing laboratory system.

Design Scheme

Design Scheme for Physicochemical Analytical Workstation

The physicochemical analytical workstation integrates a fully automated pretreatment platform with a hybrid robotic system (Figure 1), providing round-the-clock and automated capabilities for forensic specimen processing and analytical screening. This system enables high-throughput identification of narcotics and toxicants through standardized workflows.

Figure 1: Integrated model of a fully automated pretreatment platform and hybrid robotic system.

The fully automated pretreatment platform incorporates modular instrumentation for essential procedures: organic solvent dispensing, vortex mixing, centrifugation, sample transfer, solvent evaporation, and volume normalization. Enhanced with collaborative robotic arms and auxiliary mechanisms, it executes precision operations including liquid handling, agitation, phase-separation extraction, concentration, and power-on/power-off maintenance (Figure 2). The hybrid robotic system demonstrates dual mobility and collaborative operation capabilities, enabling bidirectional signal interfacing with both the pretreatment platform and three categories of six analytical instruments. Analytical result interpretation is achieved through proprietary spectral deconvolution algorithms, facilitating automated screening of over 200 narcotics and toxicants.

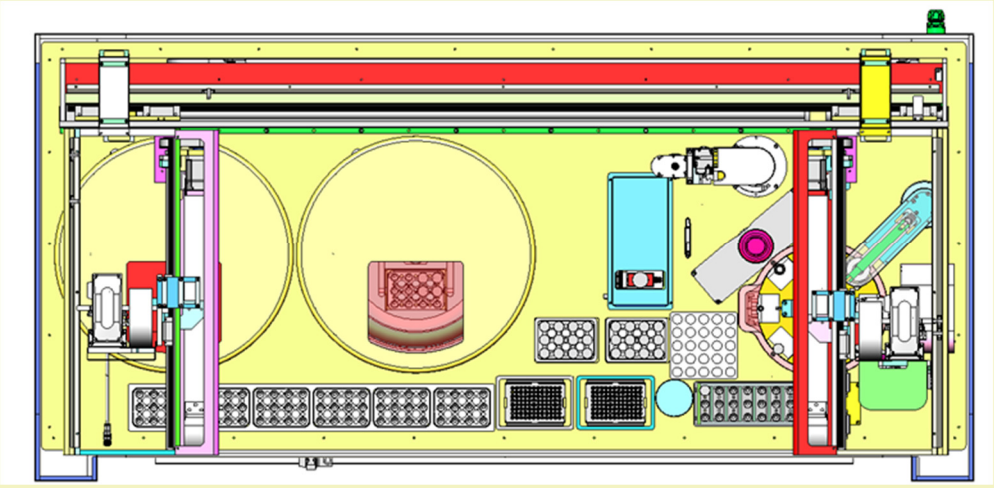


Figure 2: Integrated schematic overhead plan view of the fully automated pretreatment platform.

Analytical Workflow Protocol

1. Specimen Pretreatment

The robotic arm executes precision aspiration of blank plasma/urine matrices spiked with dual internal standard mixtures, followed by rigorous vortex homogenization. Subsequently, organic solvent is introduced for phase separation via mechanical agitation. The mixture undergoes cryogenic centrifugation, after which the supernatant is quantitatively transferred to a microcentrifuge tube for solvent evaporation under nitrogen stream. The residue is reconstituted with a metered volume of chromatographic-grade organic solvent through vortex mixing, followed by aliquot transfer into pre-labeled autosampler vials.

2. Automated Sample Analysis

Upon navigating along the predefined trajectory, the robotic manipulator transports the sample tray into the designated instrument chamber. Utilizing vacuum grippers with force feedback control, the system precisely positions test specimens onto the autosampler tray coordinates. Through touch panel interaction, a bidirectional signal handshake is established, initiating automated analytical operations in accordance with preprogrammed settings.

3. Outcome Assessment

Upon completion of instrument operation, the robotic system transfers the analyzed samples to a controlled storage compartment, initiates data file access via integrated software for spectral analysis and automated database matching, generates and saves operational logs and screening reports, produces and prints finalized documentation.

Construction Specifications for Laboratory Renovation

Structural Modifications

The newly constructed laboratory measured 8.5 meters in length, 5.5 meters in width, and 2.9 meters in height. The flooring system employed 600mm×600mm environmentally stabilized PVC resin raised access flooring panels arranged in modular grid configurations. The assembly was elevated 200mm above the substructure, providing dedicated utility raceways for concealed mechanical/electrical distribution. The laboratory envelope system integrated 50mm-thick custom-fabricated rock wool acoustic dampening sandwich panels with 0.5mm gauge stainless steel cladding. Core specifications included: mineral wool density ≥100 kg/m³, certified fire resistance exceeding 60 minutes, and three-sided wall assemblies utilizing dual-pane acoustic insulation tempered glass with cleanroom-grade white powder-coated aluminum alloy framing profiles.

Two High-Efficiency Particulate Air (HEPA) filter supply vents were installed on the laboratory ceiling, each with a rated airflow of 1000 m³/h. LED panel lighting fixtures and surveillance cameras were installed. A supporting air-conditioning system was integrated, comprising a ducted air-cooled unit with an airflow capacity of 2000 m³/h, cooling capacity of 7.5 kW, and heating capacity of 8 kW. Additionally, a set of 220V floor-mounted power outlets, powered by an uninterruptible power supply (UPS), was installed with a total connected load of 8 kW (Figure 3).

Figure 3: View of an intelligent fully automated physicochemical analytical laboratory.

Design of Core Components

1. Transport Robotic Arm and Pipette Robotic Arm

The transport robotic arm employed a gripping mechanism capable of grasping and relocating 4×3 test tube racks (Figure 4), while the pipette robotic arm integrated pipetting functionality. The latter was programmable for precise liquid transfer operations, enabling accurate aspiration and dispensing of fluid volumes up to 1 mL through quantitative parameter configuration.

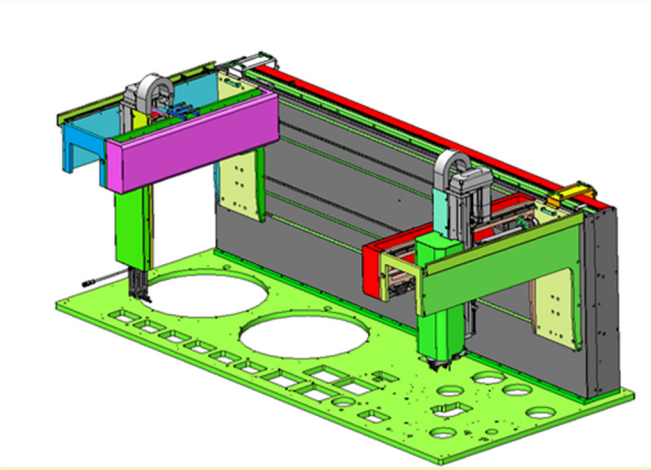


Figure 4: Transport robotic arm and pipette robotic arm.

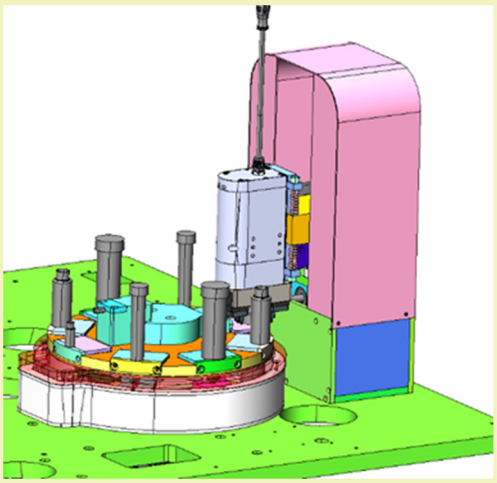


Figure 5: Integrated schematic of test tube capping/decapping device.

2. Sample Vessel Capping/Decapping Device

The capping/decapping system comprised a motorized rotary annular platform serving as the base. This platform was integrated with diameter-specific retractable apertures distributed along its circumference. Through controlled rotation, the platform aligned apertures matching the dimensions of target test tubes or sample vials beneath the capping robotic arm. Following placement of the vessel into an expanded aperture, the aperture contracted to secure the vessel via radial compression. The robotic arm's dual-action gripper simultaneously engaged the cap and applies programmable rotational torque, thereby completing automated cap manipulation (Figure 5).

3. Organic-Aqueous Phase Interface Recognition System

Following organic solvent extraction and centrifugation of the sample, the robotic arm transported the test tube to the phase interface recognition zone. A miniature CCD camera captured lateral images of the tube, which were transmitted to a central processor for real-time image analysis. The organic-aqueous phase separation interface height was algorithmically identified through grayscale gradient detection. Subsequently, the liquid-handling robotic arm precisely aspirated the organic phase supernatant above the quantified interface level.

Electromechanical Control Design, Fabrication, and Software Development

In compliance with national and forensic regulatory standards for analytical workflows, comprehensive technical documentation was developed, including assembly schematics, system block diagrams, circuit schematics, wiring layouts, and general arrangement plans. Concurrently, control algorithms for auxiliary mechanisms and integrated process automation software were programmed with modular architecture to ensure scalability and compliance with standards.

Electromechanical Integration and Commissioning

After individual functional validation of experimental modules, system-wide commissioning was executed according to forensic sample processing protocols. Device stability metrics were quantified through accelerated lifecycle testing, establishing optimal control ranges for critical operational parameters during sustained operation.

Pilot Operation and Validation

Following completion of experimental module assembly and electromechanical synchronization validation, a standardized protocol-driven blank-run operation was initially executed to verify baseline system functionality. Upon achieving operational stability, spiked positive controls were introduced for analytical validation. Parallel manual control experiments were conducted under guidelines to assess the automated platform's performance metrics, including recovery rate, detection sensitivity and intra/intra-day precision.

Discussion

As a key business department of the Public Security Bureau, criminal technology has always been at the forefront of technological innovation in public security work. In particular, physical and chemical analysis technology has been constantly innovating and transforming, with the detection capability and level advancing rapidly. How to meet the new practical requirements of criminal technology is posing increasingly severe pressure for the physical and chemical inspection profession.

To deeply implement the principle of "Primacy to the people, Preeminence to life", and in accordance with the principle of "mandatory toxicological screening" [2], Jiangsu province has formulated 22 standard methods and defined a screening scope of 240 types of toxic (drug) substances. For the standard process-based inspection work of 3,500 to 4,000 biological samples each year, introducing mobile and collaborative composite robots into the sample processing, transporting and loading, and instrument analysis stages can replace the complex manual operations. At the same time, it can achieve fine and standardized inspection processes, scientific and standardized inspection methods, safe and pollution-free inspection environments, accurate and traceable inspection results, as well as rapid and efficient inspection from the aspects of personnel, machines, materials, methods, and environment.

This project aims to develop an all-in-one fully automatic pretreatment platform integrating liquid transporting, shaking, centrifugation, extraction, evaporation, and volume adjustment to achieve high-throughput standardized extraction of evidence samples. The establishment of this laboratory will achieve breakthroughs in the application of fully automated intelligent technologies in the field of physical and chemical testing and identification. Simultaneously, it provides feasible technical references for the effective advancement and comprehensive implementation of "mandatory toxicological screening" coverage for biological samples in abnormal death cases, ensuring thorough toxicological analysis in such forensic investigations.

Firstly, it greatly enhances the automation level of toxic (drug) substance analysis and screening in biological samples. Within a closed laboratory, a fully automated pretreatment platform equipped with a composite robot replaces manual operations, completely eliminates errors and mistakes caused by human factors. It minimizes the possibility of cross-contamination and other uncertainties that affect the test results, allowing the standardization and accuracy of the test results to be maximally reflected.

Secondly, it saves manpower, improves efficiency, and protects the safety of laboratory personnel. After the test procedures are set and saved through the terminal, a single click can activate the entire process, which not only liberates manpower but also removes the technical barriers for users. Even under high-load operation, it can ensure the accuracy of test results, achieving a state where the machine operates continuously while personnel rest. At the same time, it significantly reduces the long-term harm of toxic and harmful chemical reagents to laboratory personnel, allowing technicians to devote more energy to professional learning, methodological research, case analysis, and other challenging tasks, thereby providing deeper and broader services to criminal investigation practices.

Finally, it can enhance the standardization management level of the laboratory. Through the work logs of the pretreatment platform and the composite robot, information at each stage of the test process can be accurately recorded without omission, such as the time of experimental steps, usage records of equipment, instruments and the operators, truly achieving a fine, standardized, and traceable test process and improving the standardization management level of the laboratory.

References:

1. *Liping Fang. Overview of the Development and Application of China's Composite Robot Technology [J]. Logistics Technology and Application, 2024, 29(12):122-126.*
2. *Ministry of Public Security. Guidelines for Public Security Organs on Criminal Investigation Techniques in Handling Abnormal Death Cases[Z]. 2025.2.18.*

Technical Article

AI-Enhanced Tools for the Forensic Chemical Analysis of Illicit Drugs, Cannabis, and NPS in Southeast Asia: A Scoping Review with Proposed Roadmap

Nieves Manuela A. Barbo, Katrina Jane D. Agustin, Yvette Kristine S. Desamito, Lyka M. Tiu, Shaila S. Seville*
Philippine Drug Enforcement Agency
**Email: nievesmanuela.barbo@pdea.gov.ph*

Abstract

This scoping review maps the current landscape of artificial intelligence (AI)-enhanced tools supporting forensic chemical analysis of illicit drugs, Cannabis, and new psychoactive substances (NPS) in Southeast Asia (SEA). Using the PRISMA-ScR framework, we systematically searched 8 databases, identifying 12 relevant studies that met inclusion criteria of AI application in forensic chemistry with SEA authorship. The selected studies span a variety of AI methods—including machine learning, deep learning, and ensemble models—applied to data from spectroscopy, imaging, sensor arrays, and GC-MS platforms. These tools demonstrated strong potential in improving speed, accuracy, and nondestructive testing capabilities for substance classification and quantification. However, challenges such as limited diversity and standardization of datasets, potential overfitting, inconsistent validation protocols, and constraints in cross-laboratory reproducibility remain. Our findings highlight the promise and current limitations of AI integration in forensic laboratories across the region. This review concludes by outlining a roadmap for leveraging AI to advance forensic chemical analysis in SEA while addressing existing limitations and future opportunities.

Keywords: artificial intelligence, machine learning, deep learning, prompt engineering, forensic chemistry, illicit drug, new psychoactive substance, Southeast Asia, chemometric

1. Introduction

Forensic chemical analysis is the application of chemical methodologies to detect, identify, and quantify substances within the legal context [1]. Forensic chemists (FCs) play a critical role in combating the global illicit drug trade. Apart from common illicit substances such as methamphetamine and Cannabis, the emergence of new psychoactive substances (NPS), often designed to circumvent existing drug laws, presents a significant and ongoing challenge to forensic laboratories [2]. Artificial intelligence (AI) offers a transformative approach to enhance the speed, accuracy, and efficiency of forensic drug analysis [3]. This review focuses on AI tools studied and used in Southeast Asia (SEA), a region facing significant challenges related to illicit drug trafficking and abuse [4]. These tools may have the potential to assist FCs in their analyses.

The economic limitations of most SEA countries often hinder FCs from accessing the advanced computer-related learning systems available in developed nations. Consequently, foundational AI concepts may not be widely integrated into forensic training. For the purposes of this review, we define AI as a field of computer science that enables machines to perform tasks typically requiring human intelligence. AI forms include the following:

- **Machine learning (ML):** Algorithms that allow computers to learn from data without explicit programming. This can involve training models to classify drugs, identify unknown compounds, or predict their properties. Common ML techniques include support vector machines (SVMs), random forests (RFs), and k-nearest neighbors [5].

- **Deep learning (DL):** DL is a subset of ML that uses artificial neural networks with multiple layers (deep neural networks) to analyze complex data. DL models can process hyperspectral imaging and mass spectrometry (MS) data [4], as well as enhance sensory analysis for E-nose, E-tongue, and E-eye [6].

- **Natural language processing (NLP):** This enables computers to understand and process human language. NLP can assist FCs without a coding background by generating scripts and automating workflows. The effectiveness of NLP models often depends on prompt engineering, which involves crafting precise inputs to maximize AI-generated outputs. By optimizing prompts, forensic analysts can enhance data-extraction accuracy and generate more relevant insights from large textual datasets [5].

- **Expert Systems:** These are computer programs designed to mimic the decision-making abilities of a human expert. FCs can apply them in automated spectral interpretation, i.e., analyzing and interpreting NMR, IR, or MS data by comparing unknown substances with vast forensic databases [5].

Figure 1 illustrates the key branches of AI as aforementioned. Other forms of AI include speech processing for text-to-speech and speech-to-text conversion, vision AI for image recognition and machine vision, planning and optimization for problem-solving strategies, and robotics for automated tasks [4].

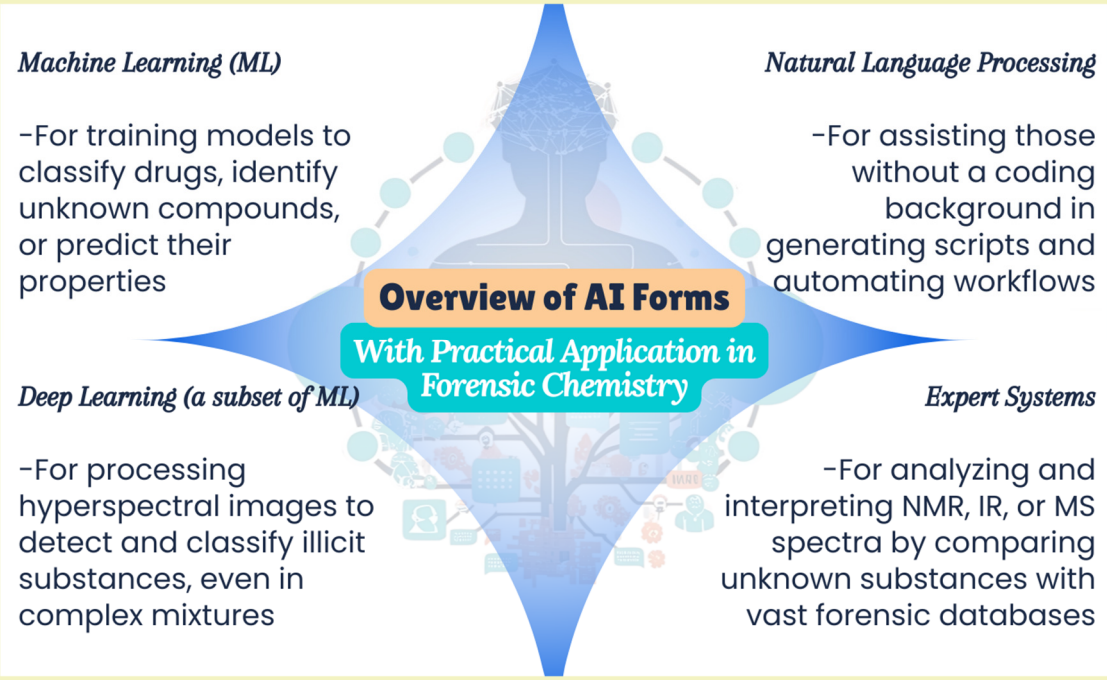


Figure 1: Overview of AI forms with practical application in forensic chemistry

Notably, AI goes beyond traditional chemometrics by its ability to learn from data in a more autonomous and flexible manner. AI can identify nonlinear relationships, automatically extract relevant features, and handle high-dimensional data, making it particularly well suited for the complexities of modern forensic analysis [7].

AI tools can assist FCs by automating their repetitive and time-consuming tasks, allowing them to focus on more complex and critical aspects of their work. One of its most significant contributions is in data preprocessing, where AI streamlines baseline correction, noise reduction, and peak alignment in spectroscopic data, minimizing manual effort. It also plays a crucial role in preliminary screening, rapidly analyzing large datasets—such as those from GC-MS—to flag potential samples of interest for further examination. Beyond speed, AI enhances pattern recognition, identifying subtle variations in spectra that may indicate the presence of specific drugs or adulterants, patterns that could otherwise go unnoticed by human analysts [8]. With the use of sensory analysis tools such as E-nose, E-tongue, and E-eye for chemical screening, forensic laboratories can also reduce their operational costs [9].

To explore the role of AI in forensic chemical analysis within SEA, we conducted a comprehensive scoping review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) framework. Compared with a systematic review, a scoping review aims to scope a body of literature, identify knowledge gaps, or to inform a future systematic review [10]. This review maps the current body of research involving AI-enhanced tools for analyzing illicit drugs, Cannabis, and new psychoactive substances (NPS) across the region. A strategic roadmap is also outlined to support the integration of AI in forensic workflows and promote the modernization of SEA forensic laboratories.

2. Methods

2.1 Protocol and Registration

This scoping review is guided by the PRISMA-ScR framework [11]. A formal protocol for this review has not been registered.

2.1.1 Research Questions (RQs)

RQ1: What AI, ML, or DL methods have been applied to the forensic chemical analysis of illicit drugs, Cannabis, and NPS by researchers affiliated with Southeast Asian institutions?

RQ2: What chemical analysis techniques (e.g., spectroscopy, chromatography, sensor arrays, etc.) are most commonly integrated with AI tools in the identified studies?

RQ3: What forensic applications or outcomes such as substance classification, quantification, or real-time detection have been achieved through these AI-enhanced methods?

RQ4: What thematic trends, methodological gaps, or research clusters emerge from the current literature, and how can these assist FCs' work and AI integration in SEA?

2.2 Eligibility Criteria

Studies were included in this review based on the following criteria:

- ✓ Research articles that applied AI tools to forensic chemical analysis.
- ✓ Studies demonstrating applications relevant to the classification, and description of illicit drugs, cannabis, and NPS.
- ✓ Application of AI in recognized techniques.
- ✓ At least one author must be affiliated with an institution based in SEA.

Exclusion criteria included the following:

- × Studies focusing on medical, enforcement, or sociocultural aspects of drug use (without direct application to chemical analysis).
- × Studies using mathematical or geometrical techniques without explicit AI methodologies.
- × Review articles that do not present original research using AI tools (unless they specifically address the potential of AI in a way relevant to the RQs).
- × Studies not related to the analysis of illicit drugs, Cannabis, or NPS.
- × Studies where no author is affiliated with a SEA institution.

2.3 Information Sources

A structured Boolean search was conducted across eight databases, namely, PubMed, Scopus, Web of Science, ScienceDirect, Google Scholar, CORE.ac.uk, ResearchGate, and Springer. Searches were conducted in March and April 2025 without date limitations. The final search yielded 612 records, with 43 duplicates removed. This left 569 titles and abstracts for screening. After applying inclusion and exclusion criteria, 12 studies were included in the final synthesis.

The following search syntax was adapted for each database except ScienceDirect: (("artificial intelligence" OR "large language" OR "natural language" OR "machine learning" OR "deep learning") AND ("methamphetamine" OR "ketamine" OR "cocaine" OR "ecstasy" OR "controlled substance" OR "controlled drug" OR "forensic chemistry" OR "illicit drug" OR "clandestine laboratory" OR "cannabis" OR "psychoactive" OR "narcotic")) AND ("Brunei" OR "Cambodia" OR "Indonesia" OR "Laos" OR "Malaysia" OR "Myanmar" OR "Philippines" OR "Singapore" OR "Thailand" OR "Vietnam" OR "Southeast Asia"). For ScienceDirect, the above syntax was modified into separate search queries because the maximum number of Boolean connectors is eight per field.

2.4 Selection of Sources of Evidence

Two reviewers independently screened titles and abstracts to identify studies for inclusion. Discrepancies were resolved through discussion. Twelve full-text articles were reviewed, with no exclusions at this stage. A PRISMA-ScR flow diagram was constructed accordingly, as shown in Figure 2.

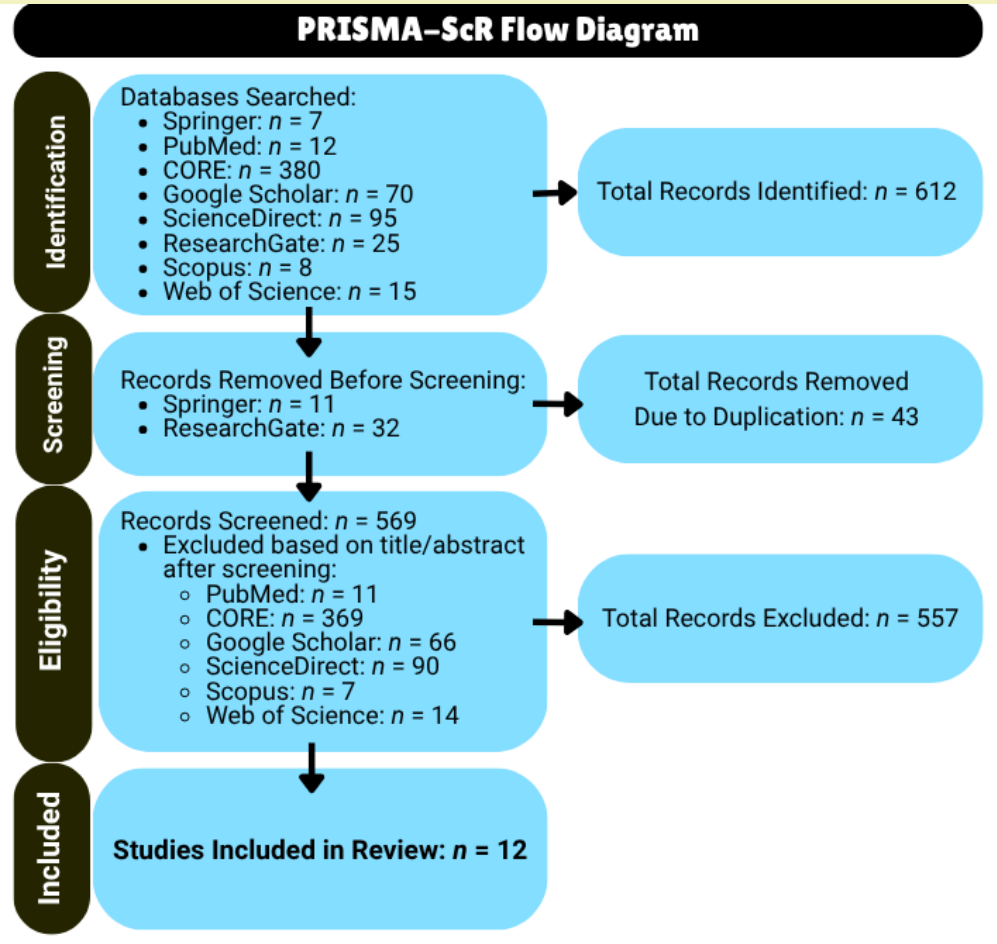


Figure 2: PRISMA-ScR flow diagram

2.5 Data-Charting Process

Data were extracted using a predefined charting form that was pilot tested prior to use. Extracted fields included the first author and year, country, AI tool/method, analytical technique, drug/s analyzed, how AI was leveraged, key findings, and limitations identified.

2.6 Synthesis of Results

The results were synthesized through a narrative approach, and the key findings are summarized in Table 1.

2.7 Critical Appraisal of Individual Sources

A critical appraisal of individual studies was also conducted, as shown in Table 2.

3. Results

A total of 12 studies met the inclusion criteria. These studies demonstrate the application of AI to various forensic tasks, including drug classification, compound quantification, GC-MS pattern recognition, and the development of portable detection tools. The AI tools range from conventional ML algorithms to DL and hybrid techniques. For each study, Table 1 summarizes the key findings, whereas Table 2 summarizes the results of critical appraisal. The detailed version of the critical appraisal is available as a Supplemental material upon reasonable request.

Table 1: Key findings of the included studies

First Author & Year	Country	AI Tool/ Method	Analytical Technique	Drug/s Analyzed	How AI Was Leveraged	Key Findings	Limitations Identified
Chang 2025 [12]	MY	K-nearest neighbor, logistic regression, support vector machine, decision tree, random forest	Attenuated Total Reflectance–Fourier Transform Infrared Spectroscopy (ATR-FTIR)	Methamphetamine, heroin, benzodiazepines	Classification of drug substances based on spectral data.	Random Forest model achieved 99.6% accuracy.	Lack of detailed information on sample selection, data collection parameters, and validation methods.
Yusof 2021 [13]	MY	Shallow one-dimensional convolutional neural network (1DCNN)	3D molecular descriptors	Amphetamine-type stimulants (ATS)	Classification of ATS drugs using molecular descriptors as input for the 1DCNN model.	1DCNN achieved comparable performance to Random Forest.	Details of the dataset of ATS drugs and the derivation of the 3D molecular descriptors are not fully clear. Validation methodology requires further scrutiny.
Rahmantyo 2019 [14]	ID	Support Vector Machine (SVM), Principal Component Analysis (PCA)	Electronic Tongue (TOMA and OA lipids)	Cannabis (pure, mixed with tea, mixed with tobacco)	Feature selection using PCA to optimize sensor array and SVM for classification.	Optimized sensor subsets (6 for cannabis-tea, 3 for cannabis-tobacco) achieved 100% accuracy with reduced running time.	Lack of detailed information on sample selection, exact validation procedure, and specific sensor array composition.
Aryati 2020 [15]	ID	Deep Learning (CNN), Machine Learning (Fingerprint Similarity-Based Clustering), Pharmacophore Modeling	2D NPS structures, Fingerprints, Physicochemical properties	Cannabinoid- and cathinone-derived compounds (New Psychoactive Substances - NPS)	Classification of NPS compounds using different AI methods and comparing their performance.	Deep learning method using fingerprints showed the best performance for classifying both types of compounds with high accuracy.	Selection criteria for the NPS database are not fully described. Detailed information on the validation methods for deep learning models is lacking.
Holmes 2020 [16]	MY	Statistical Machine Learning	Near-Infrared Hyperspectral Reflectance Imaging	*Cannabis sativa* (Flowers, Stems, Leaves)	Classification of different parts of the cannabis plant based on spectral data.	Statistical machine learning with hyperspectral imaging can effectively classify cannabis plant parts.	Data preprocessing steps, specific machine learning models used, and rigor of validation process need examination in the full text.
Chumchu 2023 [17]	TH	Not applicable (Dataset paper)	Image dataset	Cannabis seeds (17 classes)	Creation of a labeled image dataset for training machine learning models.	Provided a dataset of 3434 high-quality images of 17 cannabis seed classes.	Potential limitations in the representativeness of seed categories and standardization of image acquisition conditions.
Hendrick 2022 [18]	ID	Artificial Neural Network (ANN)	Portable Electronic Nose	Dry Cannabis	Classification of dry cannabis aroma using an ANN trained on sensor data.	ANN achieved 96% accuracy in predicting dry cannabis aroma.	Sensor array details, dataset creation for ANN training, model architecture, training process, and system validation need examination in the full text.
Kurnianingsih 2023 [19]	ID	Ensemble Learning (Soft Voting), ANFIS, Random Forest, MLP, k-NN, SVM	Electronic Nose (custom hardware)	Methamphetamine (in urine)	Combining outputs of multiple classifiers using soft voting to improve detection accuracy.	Proposed ensemble learning approach aimed to improve methamphetamine detection in urine.	Details regarding sample collection, electronic nose sensors, data acquisition protocol, and ensemble model validation require further examination.

Table 1: Key findings of the included studies (cont'd)

First Author & Year	Country	AI Tool/ Method	Analytical Technique	Drug/s Analyzed	How AI Was Leveraged	Key Findings	Limitations Identified
Ooi 2023 [20]	MY	Gaussian Process Regression, Least Angle Regression, CCA, EnCCA, PLS, RPLS, PLSFS	Near-Infrared Hyperspectral Imaging	Cannabidiolic Acid (CBDA) content in *Cannabis sativa* L.	Estimation of CBDA content using various machine learning regression techniques on spectral data.	Gaussian Process Regression performed best; PLSFS offered interpretability.	Experimental setup for HSI, reference CBDA measurements, specific ML model parameters, and validation procedures need examination in the full text.
Aryati 2019 [21]	ID	Machine Learning	Spectral Imaging	Marijuana Plants (*Cannabis sativa* L.)	Review of methods and techniques for cannabis plant identification.	Provided an overview of the field.	Lack of detail on the systematicity of the literature search and synthesis methods raises concerns about potential bias.
Wong 2023 [22]	SG	Artificial Neural Network (ANN), Convolutional Neural Network (CNN), Balanced Random Forest (BRF)	Gas Chromatography –Mass Spectrometry (GC–MS)	Novel Psychoactive Substances (NPS) - cathinones, cannabinoids, phenethylamines, piperazines, tryptamines, fentanyl, and other unrelated compounds	Classification of unknown NPS based on GC-MS data.	Balanced Random Forest model achieved a macro-F1 score of ~0.9.	Model's ability to generalize to truly novel NPS with significantly different structures might require further investigation.
Ooi 2023 [23]	MY	Canonical Correlation Analysis (CCA), Ensemble CCA (EnCCA), Partial Least Squares Regression (PLS), Regularized PLS (RPLS), PLS with Feature Selection (PLSFS)	Near-Infrared Hyperspectral Imaging	Tetrahydrocannabinolic Acid (THCA) concentration in *Cannabis sativa* L.	Estimation of THCA concentration using sparse and reproducible machine learning on spectral data.	PLSFS method led to reproducible models with small feature sets.	Lack of detailed information on sample selection, HSI data acquisition parameters, and specific quantitative results.

*Note (country abbreviations): **ID** - Indonesia; **MY** - Malaysia; **SG** - Singapore; **TH** - Thailand.*

Table 2: Critical appraisal of included studies

First Author & Year	Overall Risk of Bias	Key Strengths	Key Limitations
Chang 2025 [12]	Moderate	Rapid, nondestructive method; high accuracy; first application to Malaysian samples	Lack of detail on sample selection, data collection parameters, and validation methodology
Yusof 2021 [13]	High	Novel use of shallow 1DCNN with 3D molecular descriptors; hyperparameter optimization	Details of the dataset not fully clear; justification for the use of specific 3D molecular descriptors requires further scrutiny
Rahmantyo 2019 [14]	Moderate	Use of electronic tongue response analysis using SVM for cannabis samples - potential for portable detection.	Lack of access to the full text to determine critical methodological details regarding sample preparation, sensor characteristics, SVM model parameters, and validation procedures.

Table 2: Critical appraisal of included studies (Cont'd)

First Author & Year	Overall Risk of Bias	Key Strengths	Key Limitations
Aryati 2020 [15]	Moderate	Direct comparison of machine learning and deep learning methods with pharmacophore modeling for classifying cannabinoid- and cathinone-derived compounds.	High reported accuracies of deep learning models necessitate careful examination of the validation strategies employed and the potential for overfitting, especially given the complexity of these models and the potential size of the datasets used.
Holmes 2020 [16]	Moderate	Novel application of NIR hyperspectral imaging. High classification accuracy. Identification of important spectral bands. Nondestructive approach.	Limited sample size. Convenience sampling. Lack of direct chemical validation. Different imaging conditions.
Chumchu 2023 [17]	Low	Creation and public availability of a dedicated image dataset of cannabis seeds specifically designed for machine learning applications.	Potential limitations in the representativeness of the selected cannabis seed varieties and the standardization of image acquisition conditions (e.g., variations in lighting and angle) should be considered when utilizing this resource for training machine learning models.
Hendrick 2022 [18]	Moderate	Novel application of e-Nose technology. Development of a portable system. Use of ANN for data analysis.	Small sample size. Insufficient detail on sample characteristics. Limited discussion of confounding factors. Lack of comparison with other detection methods.
Kurnianingsih 2023 [19]	Moderate	Proposal of a novel ensemble learning approach using soft voting to enhance the accuracy of methamphetamine detection in urine.	Details regarding sample collection criteria and the representativeness of the samples are lacking. Specifics of the electronic nose sensors and data acquisition protocol are not provided. The validation methodology and detailed performance results for the ensemble model require further examination.
Ooi 2023 [20]	Moderate	Systematic evaluation of regression techniques. Use of robust reference method (LCMS). Detailed analysis of model performance. Demonstrates potential of hyperspectral imaging.	Relatively small sample size. Variability of CBDA within samples. Limited generalizability to high-THCA varieties.
Aryati 2019 [21]	High	Provides a review of the existing literature on the identification of marijuana plants (*Cannabis sativa* L.) using spectral imaging and machine learning techniques.	The lack of detailed information regarding the systematicity of the literature search strategy and the methods used for synthesizing the findings from the included studies raises significant concerns about potential bias in the comprehensiveness and objectivity of the review.
Wong 2023 [22]	Low	Development of machine learning models for screening unknown NPS utilizing only GC-MS data.	While the dataset includes a diverse selection of compounds, the model's ability to generalize to truly novel NPS with significantly different structures might require further investigation.

Table 2: Critical appraisal of included studies (Cont'd)

First Author & Year	Overall Risk of Bias	Key Strengths	Key Limitations
Ooi 2023 [23]	High	Focus on developing a sparse and reproducible machine learning technique using near-infrared hyperspectral imaging for the estimation of tetrahydrocannabinolic acid (THCA) concentration in *Cannabis sativa* L.	Lack of detailed information on sample selection, HSI data acquisition, and specific quantitative results on model performance and reproducibility in the available snippets.

4. Discussion

This scoping review identified and examined 12 studies that applied AI to the forensic chemical analysis of illicit drugs, Cannabis, and NPS in SEA. The studies varied widely in their methodological approaches, target substances, and technological platforms, reflecting the diverse and evolving landscape of AI applications in regional forensic science.

Several studies demonstrated the potential of ML and DL models to significantly improve the accuracy and efficiency of substance classification. Spectroscopic techniques such as ATR-FTIR and near-infrared hyperspectral imaging were frequently coupled with supervised learning algorithms like RF, SVM, and sparse alternating decision trees, achieving high classification accuracies (often exceeding 95%). These models were applied to methamphetamine, Cannabis components, CBDA/THCA quantification, and NPS categorization. Ensemble methods and regression models further expanded AI's utility for quantifying drug content and interpreting spectral or sensor-based data.

Studies involving E-tongue and E-nose technologies, combined with ML, illustrated the feasibility of portable, real-time detection systems for Cannabis and methamphetamine, thereby providing viable alternatives to traditional methods such as GC-MS and LC-MS. Meanwhile, dataset-centric studies introduced standardized image repositories for Cannabis seeds, supporting future AI research.

Despite these promising developments, common limitations emerged. Many studies were constrained by small or region-specific datasets, with limited sample diversity and insufficient detail on sampling methods, increasing the risk of selection bias. Several lacked robust validation procedures or did not fully report metrics such as precision, recall, and cross-validation strategies, raising concerns about overfitting and generalizability. Moreover, few studies addressed the operational integration of AI tools into real-world forensic workflows, limiting their immediate applicability across laboratories with varying levels of technical capacity.

Collectively, these findings suggest that AI holds great promise for transforming forensic chemical analysis in Southeast Asia. However, substantial work remains to overcome methodological, infrastructural, and standardization barriers.

5. Proposed Roadmap for Leveraging AI Tools for Illicit Drug, Cannabis, and NPS Analyses in SEA

To fully realize AI's transformative potential in forensic chemical analysis, the following recommendations are proposed.

Phase 1: Standardize Data Collection and Validation Protocols

Uniform methodologies for sample collection, data acquisition, and cross-validation should be established to improve reproducibility and facilitate model comparison.

Phase 2: Develop Regional Data Repositories

Creating open-access, diverse datasets—spanning various drug types, formulations, and analytical techniques—will enhance model generalizability and support cross-border collaboration.

Phase 3: Promote Interdisciplinary and Cross-Laboratory Collaborations
Collaboration between forensic scientists, data scientists, and regulatory bodies is essential to design AI systems that are technically robust and contextually appropriate for SEA forensic laboratories.

Phase 4: Invest in Capacity Building and Infrastructure
Training programs, funding for instrumentation, and institutional support are needed to bridge the gap between research and practice, especially in low-resource settings.

Phase 5: Establish Ethical and Regulatory Frameworks
Guidelines should be developed to ensure ethical deployment, data privacy, and legal admissibility of AI-generated forensic results.

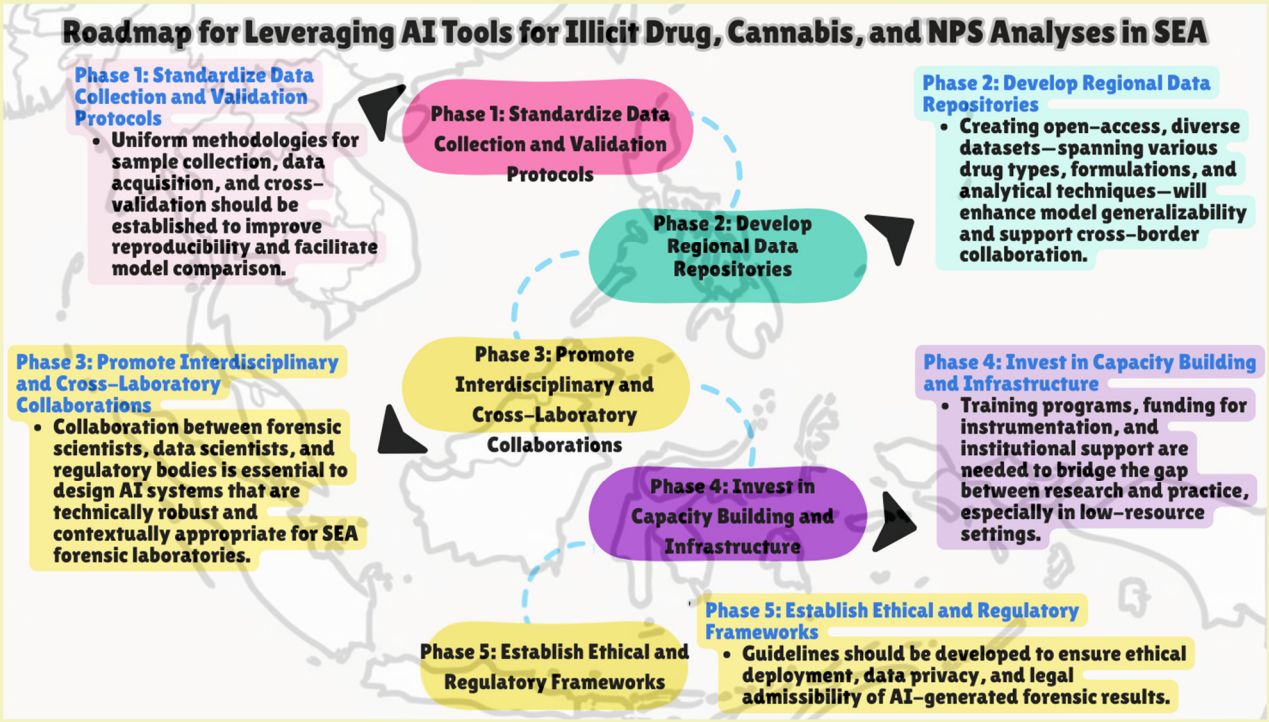


Figure 3: Roadmap for Leveraging AI Tools for Illicit Drug, Cannabis, and NPS Analyses in SEA

5. Conclusion

This review highlights the emerging but uneven application of AI tools for forensic chemical analysis in SEA. The reviewed studies showcase significant potential for enhancing analytical accuracy, reducing processing times, and enabling portable, nondestructive testing for various controlled substances. However, the current landscape is marked by fragmented efforts, varying methodological rigor, and a lack of standardized practices.

6. Acknowledgements

The authors gratefully acknowledge Ms. Aleah Marie P. Oseña and Ms. Jasmine Grace A. Operania for providing access to some screened journals.

References:

1. *Houck MM, Siegel JA. Fundamentals of forensic science. 3rd ed. San Diego: Academic Press; 2015.*
2. *Sajwani HS. The dilemma of new psychoactive substances: A growing threat. Saudi Pharm J. 2023 Jan 13;31(3):348–350. doi: 10.1016/j.jsps.2023.01.002.*
3. *Elhag-Idris G. The integration of AI in forensic chemistry [Internet]. LinkedIn; [date unknown] [cited 2025 Mar 31]. Available from: https://www.linkedin.com/pulse/integration-ai-forensic-chemistry-gamal-elhag-idris-ovtyc/ .*

References (cont'd):

4. *United Nations Office on Drugs and Crime (UNODC). Synthetic drugs in East and Southeast Asia 2024 [Internet]. Vienna: UNODC; 2024 [cited 2025 Mar 31]. Available from: https://www.unodc.org/roseap/uploads/documents/Publications/2024/Synthetic_Drugs_in_East_and_Southeast_Asia_2024.pdf.*
5. *Kleinstreuer N, Hartung T. Artificial intelligence (AI)-it's the end of the tox as we know it (and I feel fine). Arch Toxicol. 2024 Mar;98(3):735-754. doi: 10.1007/s00204-023-03666-2. Epub 2024 Jan 20.*
6. *Tan J, Xu J. Applications of electronic nose (e-nose) and electronic tongue (e-tongue) in food quality-related properties determination: A review. Artif Intell Agric. 2020;4:104-115. doi:10.1016/j.aiia.2020.06.003.*
7. *Md Ghazi MG, Lee LC, Sino H, Abdul Halim MI. Review of contemporary chemometric strategies applied on preparing GC –MS data in forensic analysis. Microchem J. 2022;181:107732.*
8. *Sivathapandi P, Krishnaswamy P, Muthusubramanian M. Advanced AI algorithms for automating data preprocessing in healthcare: optimizing data quality and reducing processing time. J Sci Technol (JST). 2022 Jul-Aug;3(4):126.*
9. *Peña-Peña ML, Marqués C, Garrido-Cardenas JA, de Armas R, Manzano-Agugliaro F. Electronic nose, electronic tongue and computer vision for food analysis: A review. Sensors (Basel). 2023;23(2):672. doi:10.3390/s23020672.*
10. *Munn Z, Peters MDJ, Stern C, Tufanaru C, McArthur A, Aromataris E. Systematic review or scoping review? Guidance for authors when choosing between a systematic or scoping review approach. BMC Med Res Methodol. 2018;18(1):143. doi:10.1186/s12874-018-0611-x.*
11. *Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. Ann Intern Med. 2018;169(7):467-473. doi: 10.7326/M18-0850.*
12. *Chang KH, Chua HN. A machine learning model for the classification of illicit drug substances with Fourier transform infrared spectroscopy. Microchem J. 2025;212:113427. doi:10.1016/j.microc.2025.113427.*
13. *Mohd Yusof N, Muda AK, Pratama SF, Carbo-Dorca R. Amphetamine-type stimulants (ATS) drug classification using shallow one-dimensional convolutional neural network. Mol Divers. 2022;26(3):1609–1619. doi:10.1007/s11030-021-10289-1.*
14. *Rahmantyo WH, Lelono D. Analisis respons sensor electronic tongue terhadap sampel ganja menggunakan support vector machine. Indones J Electron Instrum Syst. 2019;9(2):141-150. doi:10.22146/ijeis.49173.*
15. *Aryati WD, Susanto GM, Siddiq M, Winarko A, Yanuar A. Application of the machine and deep learning methods for the classification of cannabinoid- and cathinone-derived compounds. Int J Appl Pharm. 2020;12(1):1–5.*
16. *Holmes WS, Ooi MP, Kuang YC, Simpkin R, Lopez-Ubiria I, Vidiella A. Classifying Cannabis sativa flowers, stems and leaves using statistical machine learning with near-infrared hyperspectral reflectance imaging. In: Proceedings of the 2020 IEEE International Instrumentation and Measurement Technology Conference (I2MTC); 2020 May 25–28; Dubrovnik, Croatia. IEEE; 2020. p. 1–6. doi:10.1109/I2MTC43012.2020.9129531.*
17. *Chumchu P, Patil K. Dataset of cannabis seeds for machine learning applications. Data Brief. 2023;47:108963. doi:10.1016/j.dib.2023.108963.*
18. *Hendrick, Humaira, Yondri S, Afdhal H, Rahmatullah D. Dry cannabis detection by using portable electronic nose. In: Proceedings of the 2022 5th International Seminar on Research of Information Technology and Intelligent Systems (ISRITI); 2022 Dec 8–9; Yogyakarta, Indonesia. IEEE; 2022. p. 378–382. doi:10.1109/ISRITI56927.2022.10053031.*
19. *Kurnianingsih K, Al Faridi HNF, Wardihani ED, Kubota N, Chin WH. Ensemble learning based on soft voting for detecting methamphetamine in urine. In: Proceedings of the 2020 IEEE World Congress on Computational Intelligence (WCCI); 2020 Jul 19–24; Glasgow, UK. IEEE; 2020. p. 1–8.*
20. *Ooi MP, Kalavally V, Kuang YC, Simpkin R, Caddie M, Nowak J, et al. On machine learning methods to estimate cannabidiolic acid content of Cannabis sativa L. from near-infrared hyperspectral imaging. In: Proceedings of the 2023 IEEE International Instrumentation and Measurement Technology Conference (I2MTC); 2023 May 22–25; Kuala Lumpur, Malaysia. IEEE; 2023. p. 1–6. doi:10.1109/I2MTC53148.2023.10175994.*
21. *Aryati WD, Winarko MS, Susanto GM, Yanuar A. Review: Identification of marijuana plants (Cannabis sativa L.) using spectral imaging and machine learning. Int J Appl Pharm. 2019;11(6):1–5.*
22. *Wong SL, Ng LT, Tan J, Pan J. Screening unknown novel psychoactive substances using GC–MS based machine learning. Forensic Chem. 2023;34:100499. doi:10.1016/j.forc.2023.100499.*
23. *Ooi MP, Holmes WS, Kuang YC, Simpkin R, Caddie M, Nowak J, et al. Sparse reproducible machine learning for near-infrared hyperspectral imaging: Estimating the tetrahydrocannabinolic acid concentration in Cannabis sativa L. Ind Crops Prod. 2023;189:116123. doi:10.1016/j.indcrop.2022.116123.*

Technical Article

An Alternative Workflow for Identification of Mitragynine in Kratom Samples

Ms. Nor Fatihah Sameon, Ms. Yasmin Syahirah Noor Azman**, Ms. Nur Farisha Aina Mohd Anazen,*
Mr. Sharani Abu Samah, Dr. Vanitha Kunalan
Department of Chemistry Malaysia
*Email: *Norfatihah@kimia.gov.my,**Yasmin.syahirah@kimia.gov.my*

Abstract

Kratom, scientifically known as Mitragyna speciosa, is an indigenous plant commonly found in countries such as Malaysia, Indonesia, and Thailand. Over the years, the abuse of this plant has been discussed and studied in many countries, as it can produce opioid and stimulant-like effects on consumers, especially when taken at uncontrolled dosages. In Malaysia, commonly seized kratom is found in leaf and beverage form, and the processing of kratom leaves and kratom products is still considered illegal due to the harm it causes. Literature study showed a variety of routine instruments have been used for qualitative analysis (e.g., Gas Chromatography-Mass Spectrometry (GC-MS)) and quantitative analysis (e.g., High-Performance Liquid Chromatography (HPLC)) for seized kratom samples (leaves and kratom beverage). However, longer and more tedious sample preparation is required before analysis using these routine instruments to obtain high throughput and optimal results. In this study, a new instrument, known as the RADIAN ASAP Direct Mass Detector was used to determine the presence of mitragynine, the active compound responsible for producing opioid and stimulant-like effects. Three different types of kratom samples (fresh leaves, brown beverage, and mixed beverage) were chosen for this study, taken from real caseworks received by the Narcotic Division, Headquarters of the Department of Chemistry Malaysia. The selected samples had been previously analyzed using GC-MS and confirmed to contain mitragynine. The findings showed that the RADIAN ASAP Direct Mass Detector was able to detect the presence of mitragynine in all three types of samples. These findings highlight a new, robust, and less time-consuming method requiring less sample preparation for the identification of mitragynine compared to commonly used methods. Thus, this method offers a promising and reliable analysis for combating the abuse of kratom in the future.

Keywords: Kratom, Mitragyna speciosa, mitragynine, RADIAN ASAP Direct Mass Detector

Introduction

Mitragyna speciosa, commonly known as ketum or kratom in Malaysia, is an indigenous plant that is usually grown in climates around Southeast Asian countries, such as Malaysia, Thailand and Indonesia [1]. In Malaysia, this plant has been consumed traditionally for more than 100 years [2] to gain the pharmacological effects [3] which are similar to opium-like effect and its ability to also mimic stimulant effect of coca plant [4]. The main purposes of this consumption are to combat the feeling of weariness and boost tolerance among the workers when working in daylight [4]. Other purposes are to treat common illnesses such as cough, fever and inflammation [2]. However, due to the concerns of its narcotic effects and uncontrolled abuse by consumers, Malaysia has listed mitragynine, the major alkaloid that is responsible for the concerning effects and constitute 12% w/w of prepared crude kratom found in Malaysia [4], in the Poison Act 1952 and Regulations.

Over the years, several routine instruments have been utilized for qualitative and quantitative interests. The most used instruments to serve these purposes are Gas-Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC) [5], Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) and Ultra Performance Liquid Chromatography (UPLC) [6]. However, it is well-known that tedious sample preparation and complex extraction are crucial to isolating the targeted mitragynine alkaloid before introducing it for analysis. Considering this drawback, this study aims to identify a method for identifying the presence of mitragynine by eliminating the sample extraction step and reducing the analysis time. Thus, a new instrument, known as the RADIAN ASAP Direct Mass Detector is chosen as a confirmatory analysis technique for mitragynine identification due to its ability to provide direct analysis without the need for sample extraction, which reduces the analysis time and the use of solvents.

Methodology

Selection of kratom

Three types of kratom samples were selected and taken from the recently received caseworks by the Narcotic Division, Headquarters of the Department of Chemistry Malaysia. The samples include fresh kratom leaves, brown kratom beverage and mixed kratom beverage (mixing of kratom beverage with other flavored beverage) as shown in Diagram 1 to Diagram 3. All three types of samples were previously analysed with GC-MS and were confirmed to contain the presence of mitragynine.

Diagram 1: Front view of fresh kratom leaves.

Diagram 2: Back view of fresh kratom leaves.

Diagram 3: Brown kratom beverage (left) and mixed kratom beverage (right).

Sample Preparation

Fresh kratom leaves

Two grams of fresh kratom leaves were cut into small pieces and ultrasonicated for 10 minutes in 25 mL distilled water. The leaves were then filtered using filter paper and the solution containing mitragynine (kratom beverage) was obtained. This sample will undergo ten replicates analysis as shown in Table 2.

Sampling procedures

All three kratom beverages (including the solution prepared from the fresh kratom leaves) were diluted with distilled water using 1:50 ratio. Mitragynine standards were prepared at 1,000 ppm to serve as a positive control and distilled water was used as a negative control check. All three samples were dipped with different glass capillaries and undergo ten cycles of dipping (each dip taking ten seconds) separately before introducing into the RADIAN ASAP Direct Mass Detector (Waters). Each cycle was run for triplicates before proceeding to the next cycle. The parameters used in this analytical method are shown in Table 1 [7].

Table 1: Parameters used in RADIAN ASAP Direct Mass Detector for mitragynine identification.

Parameter	Setting
Ionization mode	ASAP+ (ionisation leads to protonation of the analyte)
Corona Current	3.1 µA
Desolvation gas and temperature	Nitrogen gas; 600 °C
Cone voltage	15, 25, 35, 50 V
Acquisition mode	Full scan MS; range m/z 50–600 (scan number) – continuum mode

Mitragynine Standards

Mitragynine standards utilized in this study is HPLC grade with > 93.0% purity and purchased from Lipomed.

Results and Discussions

Based on the results obtained, fresh leave (L) was positively contained mitragynine alkaloid with the match score value ranging from 927-945 (highest being 1000) as portrayed in Table 2. The intensity of the reading was the highest compared to the mixed beverage (WB) in Table 3 (also contained the presence of caffeine), which showed the second highest intensity of mitragynine and having a match score value between 854-960. While for brown beverage (WA), a match score ranging from 834-901 was identified as shown in Table 4. All the results acquired were positively correlated with the results obtained from GC-MS (theoretical highest quality 100) as shown in Table 2 to Table 4.

Table 2: Comparison of GC-MS results with RADIAN ASAP Direct Mass Detector match score values from kratom leaves (L).

Sample L				
Sample	**GC-MS result**		**RADIAN ASAP Direct Mass Detector**	
	Alkaloid	Quality	Alkaloid	Match score value
L1	Mitragynine	92	Mitragynine	939-945
L2			Mitragynine	938-943
L3			Mitragynine	931-937
L4			Mitragynine	937-943
L5			Mitragynine	927-941
L6			Mitragynine	938-943
L7			Mitragynine	929-940
L8			Mitragynine	936-943
L9			Mitragynine	930-939
L10			Mitragynine	941-945

Table 3: Comparison of GC-MS results with RADIAN ASAP Direct Mass Detector match score values from mixed kratom beverage (WB).

Sample WB				
Sample	**GC-MS result**		**RADIAN ASAP Direct Mass Detector**	
	Alkaloid	Quality	Alkaloid	Match score value
WB1	Mitragynine	95	Mitragynine	870-876
WB2			Mitragynine	917-933
WB3			Mitragynine	903-908
WB4			Mitragynine	854-877
WB5			Mitragynine	889-910
WB6			Mitragynine	863-906
WB7			Mitragynine	892-916
WB8			Mitragynine	859-864
WB9			Mitragynine	884-960
WB10			Mitragynine	901-921

Table 4: Comparison of GC-MS results with RADIAN ASAP Direct Mass Detector match score values from brown kratom beverage (WA).

Sample WA				
Sample	**GC-MS result**		**RADIAN ASAP Direct Mass Detector**	
	Alkaloid	Quality	Alkaloid	Match score value
WA1	Mitragynine	99	Mitragynine	870-879
WA2			Mitragynine	856-861
WA3			Mitragynine	884-886
WA4			Mitragynine	852-887
WA5			Mitragynine	848-860
WA6			Mitragynine	834-880
WA7			Mitragynine	881-901
WA8			Mitragynine	883-893
WA9			Mitragynine	843-881
WA10			Mitragynine	856-875

Furthermore, this study focuses on identifying three regions of interest for mitragynine identification and peaks with a match score value of 800 and above is deemed to be acceptable. In order to verify the identification of mitragynine in the samples, comparison of mitragynine standards was performed with all selected samples. Example of library matching of mitragynine alkaloid for L beverage and mitragynine standards are portrayed in Diagram 4 and Diagram 5, respectively. The match score is a critical parameter used to assess the degree of similarity between the detected mass spectrum of a compound in the sample and the reference library spectrum. LiveID library matching software in this instrument was used to process all the analysed data by comparing the sample spectral data against a prepared mitragynine standard reference library using a reverse fit model [8]. From the processing, an average match score will be produced considering all four cone voltages (15, 25, 35, 50) [8]. When the values are close to 1000, the analyte of interest is highly likely present in the sample and vice versa [7]. The intensity of the compound peak can be varied depending on several factors, including the number of dips introduced to the glass capillary, the duration of analyte exposure in seconds, and the dip sampling depth. In this study, it was found that higher number of dips increased the amount of mitragynine introduced into the system, resulting in a stronger signal and higher match score. Similarly, longer exposure times increase the chance for the mitragynine alkaloid to attach to the capillary surface and subsequently, enhance its detectability. Despite this, dilution of the samples was still performed as the signal reading and match score showed poor quality value when directly analysed the kratom beverages. These findings highlight the importance of standardizing these parameters to ensure robust and reproducible results.

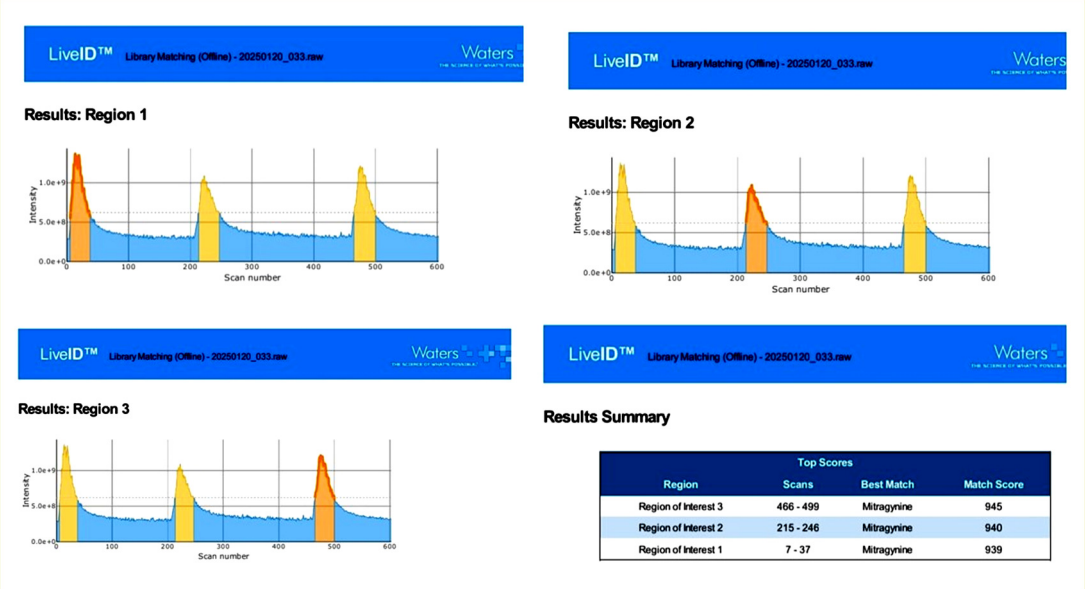


Diagram 4: Example of kratom leaves (L) match score summary analyzed using RADIAN ASAP Direct Mass Detector.

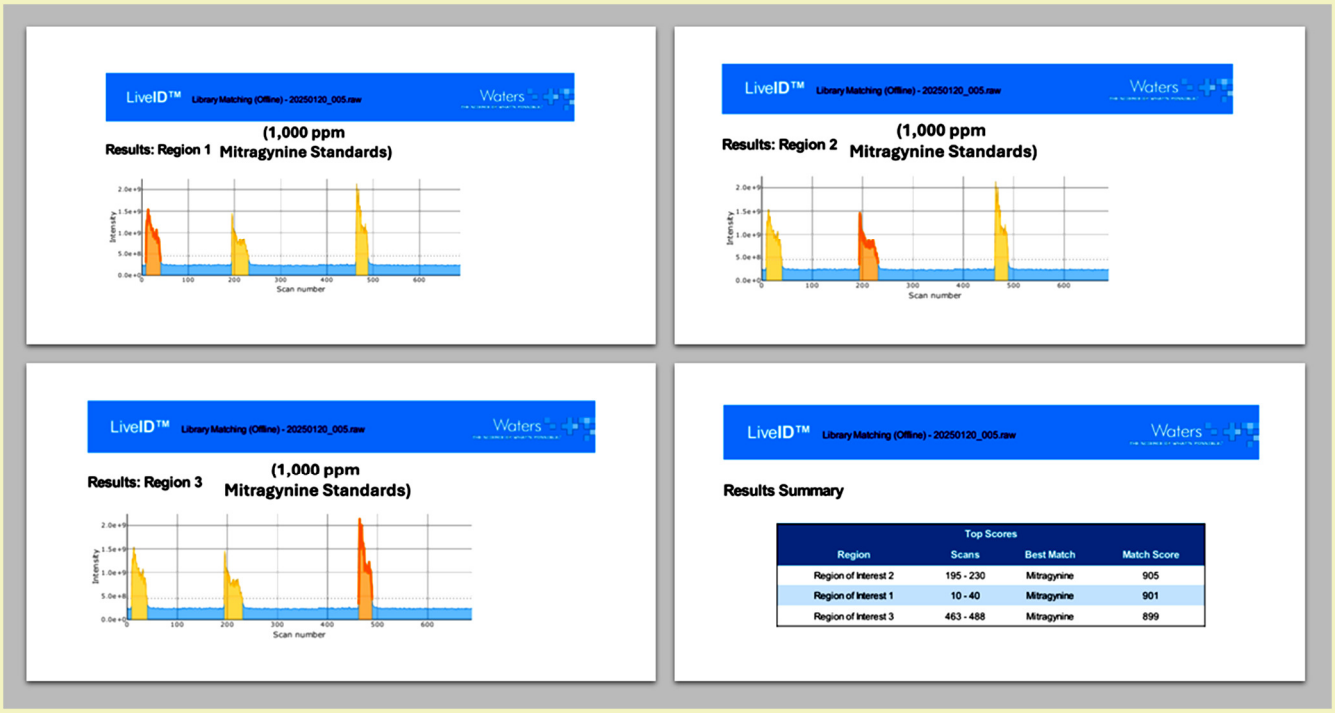


Diagram 5: Example of mitragynine standards match score summary analyzed using RADIAN ASAP Direct Mass Detector.

Preliminary analysis had been previously performed to identify the limit of detection (LOD) of mitragynine analyte in RADIAN ASAP Direct Mass Detector. Six different mitragynine standards (1000 ppm, 100 ppm, 10 ppm, 1 ppm, 0.1 ppm, 0.01 ppm) were prepared and analyzed. It was observed that the instrument was unable to detect the presence of mitragynine at 0.1 ppm and 0.01 ppm concentrations, which highlights its limitation in detecting the presence of mitragynine at trace concentrations.

Conclusion

This study introduced a new, simple, less time-consuming method with no extraction step required and almost no solvent usage involved in qualitatively detecting the presence of mitragynine alkaloid in kratom plant. This method successfully identifies mitragynine alkaloid in a variety of kratom samples commonly received by the Narcotic Division, even at low levels. It is highly recommended to design standardized sampling parameters to achieve the optimum output and reproducible results. This method will be highly useful to the researchers and practitioners, especially in Narcotic Division in providing fast and confirmatory results to cater the high volume of kratom samples received by the Division.

Bibliography:

1. *Voelker, S.E.; Kern, S.E.; Falconer, T.M. et al. Journal of Pharmaceutical and Biomedical Analysis 201 (2021) 114104. doi.org/10.1016/j.jpba.2021.1141.*
2. *Tobacyk, J. P. (2022). Qualitative content analysis of public responses to an FDA inquiry on the impact of scheduling changes to kratom. International Journal of Drug Policy, 108, 103817.*
3. *Smith, K. D.-R. (2022). Kratom use as more than a "self-treatment". The American jurna of drug and alcohol abuse, 48(6), 684-694.*
4. *Takayama, H. (2004). Chemistry and Pharmacology of Analgesic Indole Alkaloids from the Rubiaceous Plant, Mitragyna speciosa. Chemical and Pharmaceutical Bulletin, 52(8), 916-928.*
5. *Hayeema, T.; Wungsintaweekul, J. Phytochemical Analysis. 2025; 36(1): 296-306. doi:10.1002/pca.3442.*
6. *Isnaeni N, Saefumillah A, Cahyana AH. Development and Validation of a Method for Detecting and Quantifying Mitragynine in Kratom Samples Using HPLC-PDA. Eruditio: Indonesia Journal of Food and Drug Safety. 2024 Oct 25;4(2):130-44.*
7. *Lee, E.; Cooper, J.; Wood, M., Waters Application Note. 2023; 720007911.*
8. *Lee, E.; Mistry, N. S.; Calton, L. J., Waters Application Note. 2024; 720008231.*

Specifications for Quality Control of Forensic Toxicology Laboratory in China

Jing Chang, Ruihua Wang, Feng Hua, Yungfeng Zhang, Ge Song, Xinxin Ren, Aihua Wang, Yingdong, Zhongshan Yu, Fanglin Wang, Yujing Luan, Bo Zou*
Institute of Forensic Science, Ministry of Public Security, People's Republic of China
**Email: changing73@126.com*

Abstract

Many standards or guidelines of quality control (QC) for forensic toxicology have been issued or published internationally. In China, Standardization Administration of the People's Republic of China (SAC) issued a standard on QC in forensic toxicology laboratory. This represents requirements and future prospects of QC.

Introduction

In judicial practice, toxicological test results play a crucial role in a poisoning case, they could be concerned with the nature of the case, the fairness of the law, and the rights and interests of the parties involved. QC is the fundamental guarantee for the results to be scientific, accurate and impartial in the forensic toxicology laboratory. As early as the 1960s, the study on QC of forensic toxicology testing began internationally [1]. Gesellschaft für Toxikologische und Forensische Chemie (GTFCH) [2] issued "Guideline for QC in forensic - toxicological analyses", SOFT/AAFS [3] issued "Forensic Toxicology Laboratory Guidelines (published in 1996, 2000, 2002, 2006, four versions)", ANSI/ASB issued a series of standards [4], The UK & Ireland Association of Forensic Toxicologists (UKIAFT) published forensic toxicology guideline [5,6], Joris Penders [7] published "Laboratory guidelines and standards in clinical and forensic toxicology" and so on. In China, Yao L. [8] firstly proposed QC measures such as standard operating procedures (SOP) adding internal standards in samples or preparing controls for monitoring the operation process, intermediate checks of instruments in 1987, then Shuo L. [9], Min S. [10], Jing C. [11], and others [12,13] have been engaged in studying the QC which gradually covers all aspects of the laboratory, such as personnel, materials, the environment, test protocols, management system, and other relevant elements. In 2023, Standardization Administration of the People's Republic of China (SAC) issued "Forensic science - Specifications for QC in toxicology laboratory" [14], which constructed a comprehensive and systematic QC specification system for forensic toxicological testing laboratories in China. The paper presents the key content of the specifications for QC and future prospects.

1. Scope

These specifications are primarily for use in the practice of forensic toxicology except for workplace drug-testing.

The key contents include requirements of resources, process, management system and safety, covering personnel, materials, environment, samples, operation processes, reports, and so on.

2. Resource Management

Resource elements include personnel, facilities, equipment and metrological traceability.

2.1 Personnel

Laboratory personnel are only the people who are engaged in forensic toxicology analysis and are regular employee, including analysts and assistant analysts. All analysts must have legal awareness, confidentiality responsibility, and professional ethics. They should master the performance of the methods, equipment used in the laboratory, receive professional training, supervision, and assessments.

The competent analysts shall have the ability to implement QC and ensure the test results, and be responsible for the issued reports. Assistant personnel should have the corresponding competency of their positions, such as operating the instrument skillfully, preforming procedures, to assist analyst in completing various tests.

There should be a competent personnel who is responsible for formulating management documents. The responsibilities include but are not limited to: determine the laboratory's scope of toxicant testing, formulate and supervise the implementation of technical files, training and assessment plans for laboratory personnel, and review documents of reports and approval to issue report.

2.2 Facilities

The laboratory should have reasonably layout, clean environment and prevention capability for fire, explosion, poisoning, pollution and so on, to ensure the health of personnel and the safety of experimental operations. It is necessary to have relatively isolated areas which meet requirements of the different functions, obvious signs and warnings to avoid cross - contamination caused by high concentration substances in samples, for example the storage areas for samples, organic solvents and reference materials, and operation areas for analytical instruments and sample extractions, respectively. Some special samples, such as drug tablets or powder, pesticides, and so on, should be provided with independent disposal and storage areas.

The temperature and humidity of the environment should be set, monitored and documented. Facilities or equipment for chemical safety protection and rescue should be equipped in the laboratory.

2.3 Equipment

The laboratory should be supplied with equipment that is required for performance of laboratory activities , including analytical instruments, reference materials, consumables , refrigerator and reagents.

Analytical instrument. The laboratory should be equipped with professional analytical instruments according to its detection capabilities. For example, to test volatile and semi-volatile stable organic compounds, gas chromatography and gas chromatography - mass spectrometers should be equipped. Documents including performance of check and acceptance, manufacturer, purchase date, should be made for every instrument. During working period, operation instruction should be developed, and maintenance, usage records, and intermediate checks should be planned. The analytical instrument should be managed and maintained by dedicated personnel to run normally. When apparatus breakdown occurs, the test results before the failure should be evaluated. If the failure affects the results, an intermediate check should be carried out after repair and recorded for traceability. It should be the same with the relocation.

Reference materials. The laboratory should use reference materials as far as possible and should inspect and record the packaging integrity, tightness, correspondence between the certificate content and the compound structure, and expiration date for the newly purchased. Reference materials shall store in accordance with the requirements of the manufacturer’s instructions. If reference materials with an expiration date of more than one year have been opened, intermediate checks should be carried out and recorded on their expiration date, storage conditions, performance indicators, etc. according to their physical and chemical properties. Once a reference material has exceeded its expiration date or deteriorated, it should be clearly marked with inspection results, date, reasons, and record of downgrade usage or disposal, etc. A file for usage of reference materials should be established. Disposal should be properly handled and recorded. When reference materials cannot be obtained, drug tablets or powder, reagents and industrial products with precise name can be selected for qualitative testing, but should be confirmed by at least one instrument that provides structural information, such as Fourier - transform infrared spectroscopy, mass spectrometry, nuclear magnetic resonance spectroscopy, X-ray diffraction, etc. The confirmation can be completed in the laboratory or other laboratories, the results should be recorded and saved.

Reagents. The laboratory should inspect the packaging integrity, tightness, instructions, and expiration date of the purchased reagents. If necessary, appropriate detection methods should be developed for verification. Reagents should be stored and categories according to their types and instructions. For example, solid reagents and liquid reagents, organic reagents and inorganic reagents should be stored independently. Highly toxic substances, flammable materials, and corrosive chemicals should be stored independently. The storage amount of

organic solvents should be controlled, especially for solvents with low boiling points, such as diethyl ether and dichloromethane. An inbound and outbound ledger should be established. There should be a ledger control system for highly toxic substances. Reagents or the prepared solutions that are no longer in use should be disposed after harmless treatment and recorded.

3. Process Management

Process management includes commission acceptance, method selection and validation/verification, samples, testing, uncertainty, validity, report, system management.

3.1 Acceptance of Client Request

The laboratory should assess whether its capabilities can meet the client's requirements. If the client's test requirements are clear, the laboratory should test according to the client's requirements. If the requirements are unclear, they should be determined through consultations between the two parties. A contract should be signed, which stipulates the storage and disposal of the samples after the test is finished.

Receiving personnel should evaluate whether the samples are suitable for the testing, especially when there are special requirements (e.g., the detection of carboxyhemoglobin and insulin). If the test results exceed the scope of the accepted testing requests, the client should be informed, and a report should be issued accordingly.

3.2 Method Selection, Validation, and Verification

The laboratory should select appropriate methods according to the testing requirements.

Standard methods which are newly introduced for verification, while non-standard methods for validation. The validation and verification parameters should meet the requirements [14] and be recorded in a form of report. If a deviation occurs during the use of a validated or verified method, the relevant parameters should be re-validated or verified, and all relevant information, including failed data, should be recorded. The report content includes the plan, sample preparation (including target analytes, concentration, matrix, etc.), instrument used, parameters, original data and storage location, personnel, and remarks (special instructions such as operation precautions).

3.3 Sample Management

The receipt of samples should be completed in the specified area under the witness of both the client and the analyst . The analyst should check whether the packaging, appearance, and labels are complete. Samples without labels or with unclear labels should be clearly remarked with the confirmation of both. Samples that cannot be clearly marked can be refused. The accepted samples should be recorded with a unique code, packaging, weight, photos and so on in the laboratory. If there are samples with potential infectivity and danger, such as syringes and darts, they should be immediately disposed and recorded in the presence of the client.

Samples should be safely stored according to their characteristics and the requirements of the testing to prevent contamination, degradation and spoilage, etc. There should be a complete chain of custody records, including information such as serial numbers, property changes, handover time, storage location, and handover personnel.

The samples should be divided into testing samples and re-testing samples to review the test results when necessary, and the testing samples should be extracted according to the selected method. The laboratory should handle the samples for disposal according to the accepted contract.

3.4 Analytical Procedures

3.4.1 Screening Test

When analyte is not clear, presumptive screening test can be carried out first for a certain type of target analytes or a technique. The screen method should define the scope and cut-off points with spiked or standard samples to monitor its effectiveness. The results from immunoassays, chromatographic or chemical test without confirmation should not be used to issue a report.

3.4.2 Qualitative Confirmation

For confirmation, it is recommended to use a combination of detection instruments that can provide molecular structure information (such as nuclear magnetic resonance, mass spectrometry, infrared spectroscopy, etc.) and instruments that can characterize chemical or physical properties (such as chromatography, ultraviolet/visible spectroscopy, etc.). The determination of positive results should conform to principles of the selected instruments. In our work, liquid chromatography - mass spectrometry (LC-MS) and gas chromatography - mass spectrometry (GC-MS) are mainly used, and the determination is based on the retention time and the diagnostic ions. The requirements are shown in Table 1 to Table 3.

Table 1. Requirements on the repeatability of the absolute retention time for LC and GC [2].

Chromatographic separation	GC	LC
Acceptable tolerance	±1%	±2.5%

Table 2. Accepted tolerances of the relative intensities of diagnostic ions in different MS techniques [2].

Relative ion intensity	GC-EI-MS	GC-CI-MS, GC-MSn, LC-MS, LC-MSn. (n>1)
>50%	20%	20%
>20%~50%	20%	25%
>10%~20%	25%	30%
≤10%	50%	50%

Table 3. Accepted tolerances of the diagnostic ions which include the precursor ion and at least secondary ion for High-Resolution Mass Spectrometry.

Mass-to-Charge Ratio	≥ 200	< 200
Accepted tolerances	5×10-6	1 mDa

Samples with positive screening results should be re-prepared and be detected with different chemical principles than those of the preliminary test for qualitative confirmation. The operation should be carried out in parallel with positive and negative QC samples. The added content (or mass concentration) of the positive control should not be higher than the detection limit or threshold of the screening method. To prevent carryover, blank solvents should be added between samples during batch processing.

3.4.3 *Quantitative Analysis*

For quantitative analysis, matrices similar to the samples should be used as blank matrices to prepare spiked samples and calibration curves. There should be at least two spiked samples, and the relative deviation (RD) of the analyte content in the samples should be ≤ 20%.

If the isotope internal standard is used for quantitative analysis, a calibration curve can be made by the standard solution containing the internal standard and the targets. For endogenous substances, quantitative analysis can be performed by the calibration curve made by adding the standards to the test samples (standard addition method).

When using a historical calibration curve, calibration samples should be used to check the calibration curve. The samples should have at least two concentrations. The low - concentration should be less than 2 - 3 times the lower limit of quantification, and the high - concentration should be higher than 80% of the upper limit of the linear range. The obtained values of the samples should be within ± 20% of the target values.

3.5 Measurement Uncertainty

The laboratory should have the ability to evaluate the measurement uncertainty associated with quantitative results reported from test methods used in toxicological analysis. Different methods and different target substances in the same method should be evaluated separately. For methods with multiple matrices, each matrix should be evaluated independently. The following elements will be considered and documented for the estimation of measurement uncertainty [16].

- Clearly define the measurand and quantitation method.
- Identify the measurement function.
- Identify the components of uncertainty.
- Quantify uncertainties for the components.
- Convert the uncertainties to the standard uncertainties and compute the combined uncertainty.
- Compute the expanded uncertainty with coverage factor
- Produce the uncertainty budget.
- Report the result.

Not all measurement processes are capable of providing a rigorous and statistically valid estimate of uncertainty. Forensic toxicologists should develop a well-reasoned and documented approach that can be justified to both the legal and accrediting communities.

3.6 Metrological traceability

Instruments and auxiliary measuring equipment which can affect the test results should be calibrated. Reference materials should be traced to the International System of Units (SI) as much as possible. If direct traceability is not possible, metrological traceability should be demonstrated through appropriate references such as certified reference materials or standard methods.

3.7 Quality Assurance Management

Quality Assurance management establishes procedures to identify and investigate unintended consequences and errors from laboratory processes or procedures, to determine the root cause, and to initiate corrective measures and improvement plans to eliminate recurrence, as appropriate. There are three measures, namely intra-laboratory QC, external QC (collaborative testing) and corrective actions.

3.7.1 *Intra-Laboratory QC*

The following is several measurements of internal QC. The laboratory can select one or more of them and record:

- Blank controls analysis.
- Blind controls analysis.
- Double samples analysis.
- Spiked sample analysis.
- Charts for QC-Samples
- Operator comparison.
- Method comparison.
- Instrument comparison.
- Reanalysis of retained samples.

The laboratory should develop a QC plan according to its routine work. The plan should cover testing, equipment, control substances or calibrators, criteria and operators. The frequency of implementation should be based on testing, personnel capabilities, analytical instruments and so on. In daily work, the analysis of blank controls and spiked samples can replace the above-mentioned analysis.

If the laboratory selects a QC chart for internal QC, it is recommended to use the X-control chart, which is also named the mean-standard-deviation control chart. Standard operating procedure (SOP) of the chart should be developed including drawing, interpretation, out-of-control criteria and measures to deal with out-of-control situations.

3.7.2 *External QC*

The laboratory should regularly carry out external QC activities such as proficiency testing or inter - laboratory comparisons and submit reports.

3.7.3 *Corrective Actions*

When abnormal results are obtained for QC samples (such as unexpected values), blank solvents (showing contamination signs), or different results are found among samples in a case, and when the quantitative results do not conform to theory or common sense, the analysis should be repeated. Moreover, the reasons for these abnormalities should be investigated, corrective actions should be developed, and all these steps should be recorded to prevent recurrence.

3.8 Report

The report should clearly and objectively detail the clients, inspection methods and results to avoid misinterpretation or misuse of the report. For qualitative results, state "detected" or "not detected" for the target substance, include the detection limit when necessary. Quantitative results must include specific values with units.

3.9 Data review and Information Management System

The laboratory should establish a data review and information management system (digital or non-digital) for collecting, processing, recording, reporting, storing and retrieving data, and develop specific documents for managing the system's access rights, security maintenance, and usage instructions.

4. Requirements for the Management System

The laboratory should establish, implement and maintain a documented management system to ensure the quality of the laboratory's inspection results and address risks and opportunities related to laboratory activities.

5. Safety Requirements

The laboratory should have a safety risk assessment procedure to identify and evaluate unknown safety hazards (such as radioactive substances, infectious biological agents, etc.), form a report based on the assessment, and eliminate or minimize risks to personnel health and environmental safety.

6. Future Prospects

With rapid technological advancements, forensic toxicology will undergo profound changes, and QC is also challenged by evolving technologies that enable more efficient, selective and faster detection. Due to the complex principles and operations of these technologies, higher capabilities, stricter standards, more rigorous data processing, and validation are demanded.

To address these challenges, QC will be refined across three key dimensions. First, to align with international standards, we must track global advancements and actively promote the Chinese specifications to boost global influence. Second, AI and intelligent data platforms will be built for data collection, analysis, and storage to improve efficiency and management. Third, QC should be extended to new testing projects, ensuring full coverage without gaps—such as AI-driven technologies, new methods or procedures. These efforts will drive forensic toxicology toward a more scientific, fair and efficient future.

References:

1. *Battista, HJ., von Meyer, L. & Wehinger, G. Quality assurance in forensic toxicological analysis. Accred Qual Assur, 1996, 1:201–206.*
2. *GTFCh Scientific Committee QC. Guideline for QC in forensic - toxicological analyses.*
3. *SOFT/AAFS. Forensic Laboratory Guidelines – 2006.*
4. *www.asbstandardsboard.org.*
5. *Elliott SP, Stephen DWS, Paterson S. The United Kingdom and Ireland association of forensic toxicologists forensic toxicology laboratory guidelines (2018) [J]. Sci Justice, 2018, 58(5): 335 - 345.*
6. *Simon Cosbey, Simon Elliott, Sue Paterson. The United Kingdom and Ireland Association of Forensic Toxicologists; establishing best practice for professional training & development in forensic toxicology (2017). Science & Justice, 2017, 57 (1): 63-71.*
7. *Joris P. and Alain V. Laboratory guidelines and standards in clinical and forensic toxicology [J]. Accreditation and Quality Assurance, 11(6):284-290.*
8. *Yao L., Wan X. Quality Management of Toxicological Analysis Work [J]. Chinese Journal of Forensic Medicine, 1987, 3: 132 - 134.*
9. *Shuo L., Jing C., Ying D., et al. Development and Current Situation of QC in Forensic Toxicological Analysis Laboratories in Forensic Science [J]. Forensic Science and Technology, 2013, 4: 3 - 7.*
10. *Min S. On the Norms of Analytical Toxicology Laboratories and Their Quality Control [J]. Chinese Journal of Forensic Sciences, 2003, 1: 36 - 39.*
11. *Jing C., Zhong S. Y., Yi H. Current Situation and Analysis of Metrological Resources for Toxicant Detection in the Field of Forensic Appraisal[J]. Metrology & Measurement Technique, 2008, 35(12): 37-39.*
12. *Hong Y. D., Ying D., Lei P. Z., et al. Application of Deuterated Internal Standards in Forensic Toxicological Analysis[J]. Chinese Journal of Forensic Medicine, 2015, 30(02): 152-155.*
13. *Ke L. X. Research on the Quality Control of Forensic Toxicology Appraisal from a Comparative Perspective[J]. Legal System Vision, 2023, (31): 85-87.*
14. *GB/T 43449-2023. forensic science - specifications for quality control in toxicology laboratory [S]. Beijing: China Standard Press, 2023.*
15. *GA/T 1649 - 2019. Forensic sciences—Specifications for validation of examination methods for toxicants [S]. Beijing: China Standard Press, 2019.*
16. *Rod G. Gullberg (2012). Measurement Uncertainty in Forensic Toxicology: Its Estimation, Reporting and Interpretation, Toxicity and Drug Testing, Prof. Bill Acree (Ed.), ISBN: 978-953-51-0004-1, InTech, Available from: http://www.intechopen.com/books/toxicity-and-drug-testing/measurement-uncertainty-in-forensictoxicology-its-estimation-reporting-and-interpretation.*

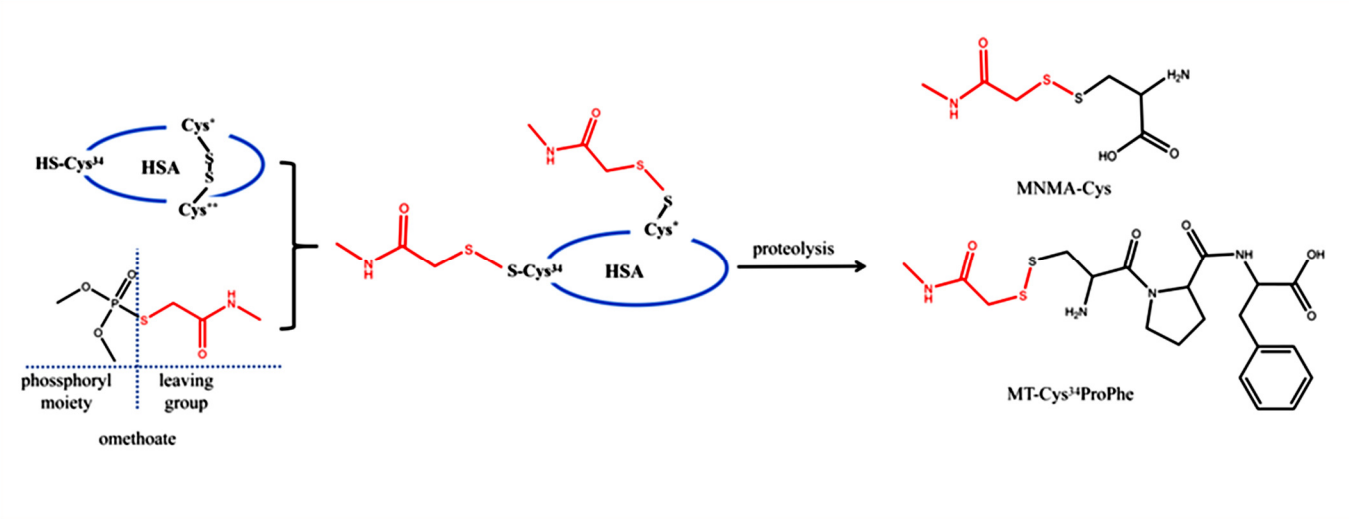
Detection of Disulfide Adducts from Human Serum Albumin Following Omethoate Exposure

*Dr. Chenglong Zhang, Prof. Ruiqin Yang**
School of Criminal Investigation, People's Public Security University of China, People's Republic of China
**Email: 20051031@ppsuc.edu.cn*

Abstract

A novel analytical method was developed for the forensic detection of omethoate (OM) poisoning in human plasma. Upon incubation of human serum albumin (HSA) with OM, disulfide adducts are formed between the thiol-containing leaving group of OM (2 - mercapto - N - methylacetamide, MNMA) and the cysteine (Cys) residue in HSA. Following proteinase K-catalyzed proteolysis, adducts of the single amino acid cysteine (MNMA-Cys), and the tripeptides cysteine-proline-phenylalanine (MNMA-Cys^{34}ProPhe) were produced. Two biomarkers were simultaneously detected Ultra Performance Liquid Chromatography - Quadrupole Exactive Orbitrap - High Resolution Mass Spectrometry (UPLC-Q Exactive Orbitrap - HRMS). Time and concentration-dependent adduct formation studies revealed that the Cys^{34} adduct exhibited optimal analytical performance, demonstrating the lowest limit of detection (20 ng/mL in plasma), and rapid formation kinetics (20 min). Finally, this novel method was applied to the plasma sample of a 49 years old deceased man who committed suicide by ingesting an insecticide containing the organophosphorus active ingredient OM. The detection of both biomarkers provided unequivocal evidence of OM poisoning, establishing the method's reliability for sensitive forensic verification.

Graphic Abstract



Upon adduction of OM with HSA, disulfide products are formed. Following proteinase K-catalyzed proteolysis, the adducts of single amino acid cysteine (MNMA-Cys) and tripeptide cysteine-proline-phenylalanine (MNMA-Cys^{34}ProPhe) are generated, which are subsequently analyzed by UPLC-Q Exactive Orbitrap - HRMS.

Introduction

Organophosphorus pesticide (OPP) intoxication represents a critical global public health challenge, accounting for approximately 200,000 fatalities annually [1]. The toxicological mechanism involves irreversible inhibition of acetylcholinesterase (AChE), leading to synaptic accumulation of acetylcholine (ACh) and consequent acute toxicity [2]. In developing countries, easy accessibility of OPPs in rural areas contributes significantly to their prevalent use in suicide cases, while their potential as chemical threat agents raises substantial security concerns. OM, a representative OPP compound, exemplifies this dilemma. Despite its production ban implemented in China since December 2023, substantial existing stockpiles continue to pose environmental and health risks. Current regulatory provisions permit legal circulation until December 2025, creating complex challenges for complete phase-out and risk mitigation.

In toxicology, clinical medicine, and forensic science, the unequivocal confirmation of OM exposure is critically important. However, bioanalytical verification of acute OM exposure in vivo presents considerable challenges due to rapid biotransformation and systemic clearance. Plasma protein adducts, particularly those exhibiting long-term stability, offer a promising alternative for retrospective exposure assessment. HSA, the most abundant plasma protein, contains multiple nucleophilic amino acid residues that serve as key targets for covalent modification by electrophilic drugs and toxins, leading to the formation of stable toxin-albumin adducts [3]. Notably, structurally analogous compounds to OM, including sulfur mustard derivatives, and V-type nerve agents, selectively react with the free thiol group of Cys^{34} in HSA, generating characteristic disulfide adducts [4]. Therefore, the aim of the present study was to identify possible disulfide-adducts of the thiolate leaving group of OM with HSA as well as their evaluation as novel biomarkers for OM poisoning. UPLC-Q Exactive Orbitrap - HRMS was employed for highly sensitive detection of the identified disulfide-adducts. Finally, a plasma sample obtained from a deceased individual with OM intoxication was analyzed to evaluate the applicability of the developed method for forensic toxicological analysis.

Materials and Methods

Biological Samples and Materials

Plasma samples were collected from 8 healthy volunteers. OM was purchased from AccuStandard® (Beijing, China). HSA was purchased from Sigma (Shanghai,China). Ultrapure water and chromatographic methanol and acetonitrile (LiChrosolv®, Germany) were used.

Incubation of neat HSA and human plasma with OM and preparation

Neat HSA solution (40 mg/mL in 50 mM NH_4HCO_3 buffer) and plasma samples were independently spiked with OM standard solution to obtain a final concentration of 5 µg/mL. All reaction mixtures were subsequently incubated at 37°C for 2 hours to produce references. Incubation mixtures were processed following the procedure described by Kranawetvogl et al. [5]. Prior to enzymatic cleavage, neat HSA solution or plasma samples (250 µL) were subjected to 3 rounds of washing with 500 µL of 50 mM NH_4HCO_3 buffer using ultrafiltration (UF) in Amicon UF devices (10 kDa molecular weight cut-off, Merc-Millipore, Darmstadt, Germany). Subsequently, proteinase K solution (500 µL, 20 mg/mL in 50 mM NH_4HCO_3 buffer) was introduced for the enzymatic cleavage reaction. This reaction was carried out at 37°C for 2 hours under gentle shaking conditions. After proteolytic cleavage, the smaller proteins present in the filtrate were precipitated. This was achieved by adding 500 µL of acetonitrile, followed by rigorous vortex mixing and centrifugation at 12000 rpm for 7 min at room temperature. The resultant 500 µL of supernatant was then dried under a gentle nitrogen stream. Finally, the dried residue was re-dissolved in 500 µL of 50 mM NH_4HCO_3 buffer.

Plasma was spiked with OM to final concentrations of 5 µg/mL (2.5 mL total volume, n = 3). Aliquots (250 µL, n = 3) were collected at 5, 10, 15, 20, 30, 45, 60, 90, and 120 min, processed per standard protocol, and analyzed via UPLC-Q Exactive Orbitrap - HRMS (PRM). The extracted ion chromatogram (XIC) peak areas (QI, Table 1) were plotted versus incubation time to determine the period for maximum adduct yield. For concentration dependence, plasma was incubated with OM (1-20 µg/mL, n = 3) and analyzed. XIC peak areas of QI (Table 1) were correlated with OM concentrations to evaluate linearity, with limits of detection (LODs) and quantification (LOQs) subsequently determined.

Instrumental conditions

Instruments used in this study were: UPLC-Q Exactive Orbitrap - HRMS (Thermo Scientific, Waltham, MA, USA). Chromatographic separation was performed on Accucore™ Biphynel column(50 mm x 2.1 mm i.d., 2.6 µm), with a total flow rate of 0.3 mL/min at 40ºC. 0.1% formic acid aqueous solution (A) and methanol (B) were used as mobile phase.

The ion source of HRMS: $HESI^+$; Acquisition mode: full scan mass spectrometry (Full MS) and parallel reaction monitoring (PRM) mode. For Full MS, resolution: 70000; AGC target: $3e^6$; maximum IT: 200 ms; scan range: 100 to 650 m/z. For PRM, resolution: 17500; AGC target: $2e^5$; maximum IT: 100 ms.

Results and Discussion

After proteinase K-catalyzed proteolysis of HSA and human plasma, each incubated separately with OM, MNMA-modified Cys-containing peptides were identified, including: the single amino acid adduct MNMA-Cys (Figure 1a) and the tripeptide adduct MNMA-Cys^{34}ProPhe (Figure 1b). Their mass spectrometric characteristics are detailed in Table 1 and 2, and Figure 2. The discovery of MNMA-Cys provides evidence that the high reactivity of MNMA can trigger the thiol-disulfide exchange reaction. As a result, MNMA-disulfide adduct can be formed even with the previously Cys-Cys disulfide-bridged residue. Due to the lack of sequence specificity, adduct formed on single Cys residue by OM cannot be assigned to specific sites. MNMA-Cys could potentially be a mixture stemming from multiple Cys residues participating in addition reactions. This non-specific adduction profile allows signal integration across different modification sites, thereby enhancing detection sensitivity.

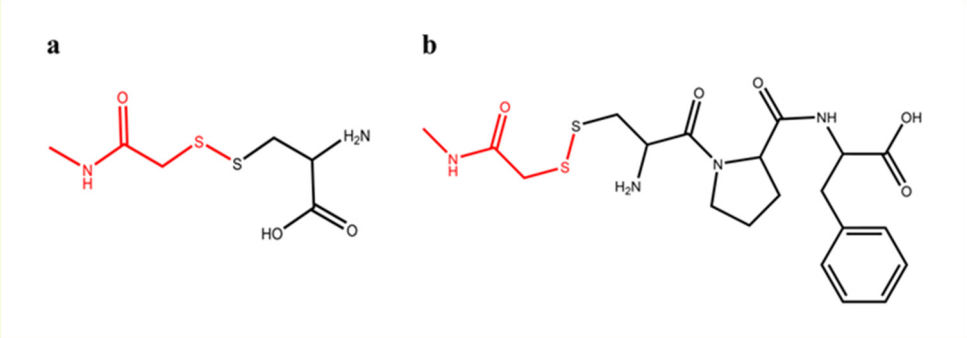


Figure 1: Chemical structures of MNMA–disulfide adducts. (a) MNMA-Cys, (b) MNMA-Cys^{34}ProPhe

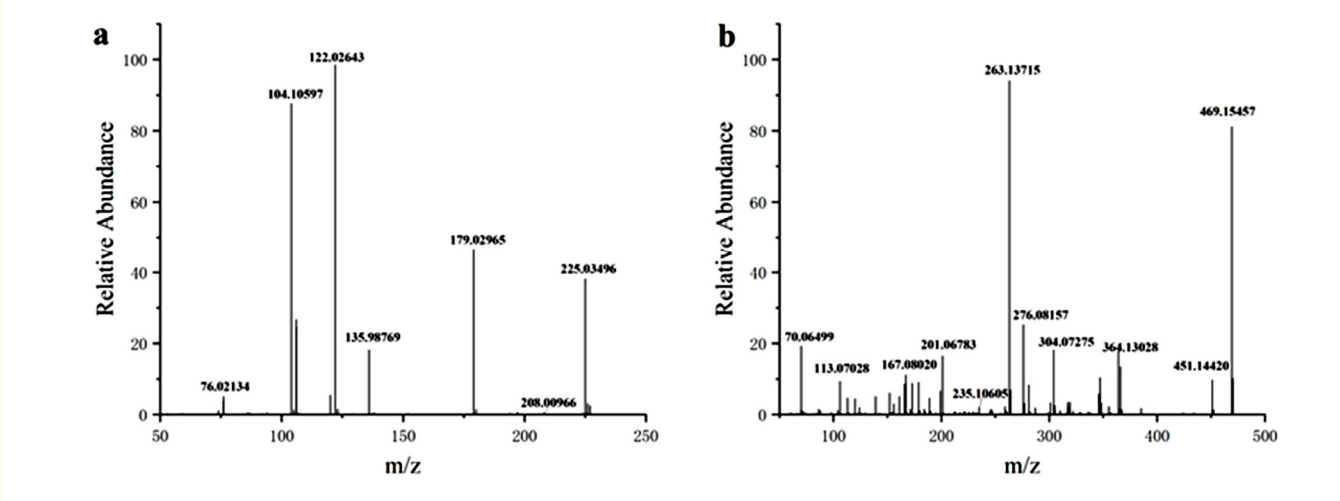


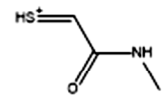
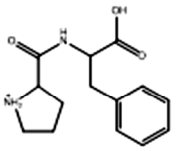
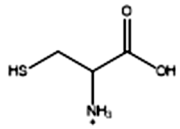
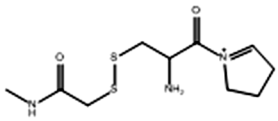
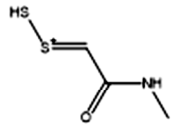
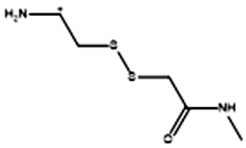
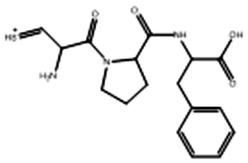
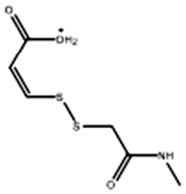
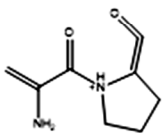
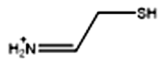
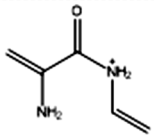
Figure 2: High-resolution product ion spectra of MNMA–disulfide adducts. (a) MNMA-Cys, (b) MNMA-Cys^{34}ProPhe

Table 1: Chromatography and mass spectrometric properties of MNMA–disulfide adducts.

Biomarker	Formula	Retention time (min)	Theoretical [a] [m/z]	Found [m/z]	Δm/z		QI [b] [m/z]
					Th	ppm	
MNMA-Cys	$C_6H_{12}N_2O_3S_2$	0.98	225.03621	225.03496	-0.00125	-5.55	122.02643
MNMA-Cys^{34}ProPhe	$C_{20}H_{28}N_4O_5S_2$	3.64	469.15739	469.15457	-0.00282	-6.01	263.13815

a Theoretical m/z of MNMA–disulfide adducts were single-protonated $[M + H]^+$.
b QI is the most intense product ion in UPLC-Q Exactive Orbitrap - HRMS (PRM) analysis.

Table 2: Characteristic product ions of the single-protonated MNMA-Cys and MNMA-Cys[34]ProPhe.

MNMA-Cys			MNMA-Cys[34]ProPhe		
Structure	Formula	Measured mass	Structure	Formula	Measured mass
	C_3H_6NOS	104.10597		$C_{14}H_{19}N_2O_3$	263.13715
	C_3H_8NO2S	122.02643		$C_{10}H_{18}N_3O_2S_2$	276.08157
	$C_3H_6NOS_2$	135.98769		C_4H_8N	70.06499
	$C_5H_{11}N_2OS_2$	179.02965		$C_{17}H_{22}N_3O_4S$	364.13068
	$C_6H_{10}NO_3S_2$	208.00966		$C_8H_{11}N_2O_2$	167.08020
	C_2H_6NS	76.02134		$C_5H_9N_2O$	113.07028

Plasma samples from eight volunteers were analyzed following the established protocol to assess endogenous OM adducts and potential interferences using UPLC-Q Exactive Orbitrap - HRMS. The analysis revealed complete absence of both endogenous OM adducts and interfering compounds in all control samples (n=8), confirming the outstanding selectivity and specificity of UPLC-Q Exactive Orbitrap - HRMS platform. The kinetics of Cys^{34} adduct formation were evaluated by monitoring MNMA-Cys^{34}ProPhe generation in plasma over time. As depicted in Figure 3, MNMA-Cys^{34}ProPhe levels increased rapidly upon OM addition, indicating an immediate reaction between OM and albumin. The concentration of MNMA-Cys^{34}ProPhe approached approximately 90% at 30 minutes, nearing the final concentration, and remained in a steady state thereafter.

The formation of MNMA-Cys took 15 minutes longer to reach the maximum yield compared to that of Cys^{34}ProPhe adducts. This observation highlights the preferential reactivity of OM with the free thiol group of Cys^{34} over other cysteine residues.

The limits of detection (LODs) and the limits of quantification (LOQs) were considered at the lowest standard with the S/N ratio of at least three and ten across all replicates. The LODs for the adducts were as follows: MNMA-Cys (20 ng/mL), and MNMA-Cys^{34}ProPhe (10 ng/mL). These LODs once more illustrated that a higher

concentration of OM was required for the transformation of intramolecular disulfide bridges into OM-disulfide adducts compared to the conversion of the free Cys[34] residue, which is attributed to the necessity of breaking the stable intramolecular Cys-Cys disulfide bridge. Therefore, MNMA-Cys[34]ProPhe appears as the most promising target to prove exposure to OM. The linearities of the two OM exposure biomarkers are presented in Table 3.

Table 3: Linear equation, linear range, R^2 , LOD, and LOQ.

Biomarker	Linear equation	linear range (ng/mL)	R^2	LOD (ng/mL)	LOQ (ng/mL)
MNMA-Cys	y = 1887.1x - 69235	50-1000	0.9944	20	50
MNMA-Cys[34]ProPhe	y = 1653x + 285756	10-1000	0.9884	10	10

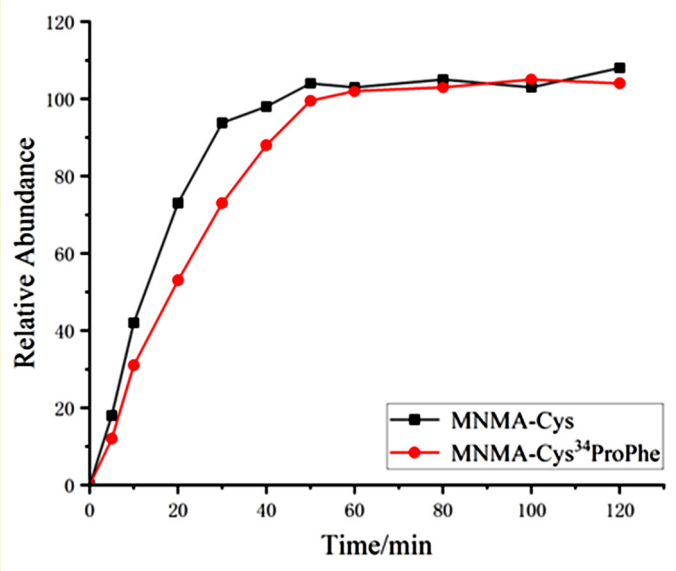


Figure 3: Time-dependent formation of MNMA-adducts in plasma.

Following three freeze-thaw cycles, MNMA-Cys and MNMA-Cys[34]ProPhe exhibited losses of 15% and 18%, respectively. In comparison, disulfide adducts derived from V-type nerve agent thiol leaving groups demonstrate superior stability under identical conditions [5], suggesting that adduct stability is structure-dependent. To preserve adducts integrity, minimizing repeated freeze-thaw cycles during plasma sample handling is recommended. The prepared plasma references for OM were maintained in the autosampler at 15°C and subjected to analysis by UPLC-Q Exactive Orbitrap - HRMS (PRM) at hourly intervals over a 24-hour period. The peak areas of QI (as presented in Table 1) served as an indicator of the biomarker concentration.

Two adduct targets exhibited stability in the autosampler at 15°C for a minimum of 24 hours, with no observable degradation trend (relative standard deviation, RSD < 3%, for each, data not shown). Consequently, these adducts are highly suitable for UPLC-Q Exactive Orbitrap - HRMS (PRM) analysis, even in extensive and time -intensive sample sets.

Application in the Real Case

On 28 December 2023, a 49 years old male was found deceased in his vehicle following intentional ingestion of an omethoate-containing pesticide. Postmortem plasma samples, collected approximately 11 hours after ingestion, were processed according to the standard analytical protocol using UPLC-Q Exactive Orbitrap - HRMS. The detection of MNMA-Cys and MNMA-Cys[34]ProPhe were verified by comparison with the chromatographic peak retention time and mass spectral characteristics of the references. The XICs of MNMA-Cys and MNMA-Cys[34]ProPhe in the blank blood sample, reference plasma incubated with OM (500 ng/mL), and real blood sample were shown in Figure 4.

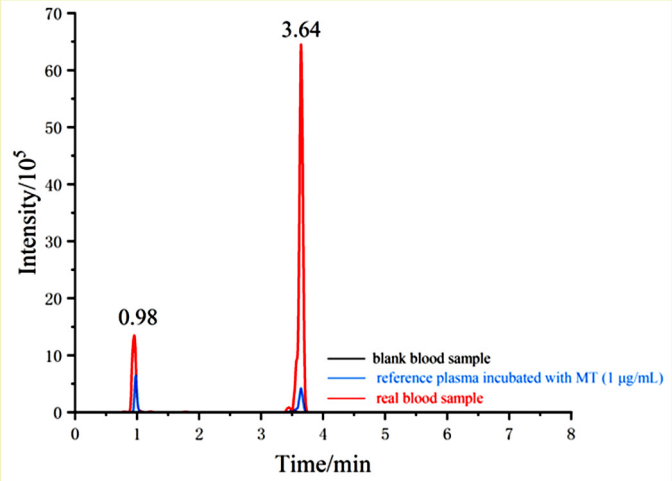


Figure 4: XICs of MNMA-Cys and MNMA-Cys[34]ProPhe in the blank blood sample, reference plasma was incubated with OM, and the real case blood sample.

Conclusion

We identified two novel plasma biomarkers, MNMA-Cys and MNMA-Cys^{34}ProPhe, capable of specifically detecting OM exposure. These novel adducts were unambiguously characterized using modern mass spectrometry techniques. Through a systematic investigation of the time and concentration dependent relationships of the two target adducts, we have determined that both adducts can be formed rapidly after OM exposure in plasma. UPLC-Q Exactive Orbitrap - HRMS allows for highly sensitive detection of these adducts (LODs ≤ 20 ng/mL). The applicability of the method was demonstrated by the successful detection of adducts in a sample from OM-poisoned patient, establishing the method as a reliable bioanalytical procedure for forensic and toxicological analysis.

References:

1. *Mew E J, Padmanathan P, Konradsen F, Eddleston M, Chang S, Philips M L, Gunnell D. The global burden of fatal self-poisoning with pesticides 2006-15: systematic review [J]. J Affect Disord, 2017,219:1574-1575.*
2. *Sembiring M H, Nursanti O, Rahmania T A. Molecular docking and toxicity studies of nerve agents against acetylcholinesterase (AChE) [J]. J Recept Signal Transduct Res, 2023, 43(1-6): 115-122.*
3. *Blum M M, Schmeier W, Dentzel M. The blistering warfare agent O-mustard (agent T) generates protein-adducts with human serum albumin useful for biomedical verification of exposure and forms intramolecular cross-links [J]. Anal Bioanal Chem, 2024, 416(26): 5791-5804.*
4. *Wang J, Lu X, Gao R, Pei C X, Wang H M. Progressive expansion of albumin adducts for organophosphorus nerve agent traceability based on single and group adduct collection [J]. Anal Bioanal Chem, 2024, 416(15): 1618-2642.*
5. *Kranawetvogl A, Worek F, Thiermann H, John H. Modification of human serum albumin by the nerve agent VX: microbore liquid chromatography/electrospray ionization high-resolution time-of-flight tandem mass spectrometry method for detection of phosphonylated tyrosine and novel cysteine containing disulfide adducts [J]. Rapid Commun Mass Spectrom, 2016, 30: 2191-2200.*

Technical Article

Development of a LC-MS/MS Method for Glyphosate, Glufosinate, and Their Metabolites in Biological Matrices – A Preliminary Study

Dr Sharmilah Kuppusami, Mr Ahmad Amirul Faiz Ahmad Tajudin, Mrs Nurul Fatihah Zahari, Mr Muhammad Rashid Shaari, Mr Ayub Ismail**
Toxicology Division, Forensic Science Analysis Centre, Department of Chemistry Malaysia
**Email: sharmilah@kimia.gov.my*

Abstract

Glyphosate and glufosinate are broad-spectrum herbicides which are frequently used in the agricultural sectors for weed control. In Malaysia, fatal intoxication cases due to these herbicides, whether accidental or intentional, have risen. Therefore, from the perspective of forensic or death investigation, a rapid and sensitive analytical method is of importance. The compounds are difficult to detect, and their pre-treatment for instrumental analysis are complicated and time-consuming. Our aim was to develop a simple and rapid quantification method for the two herbicides and their metabolite with liquid chromatography-tandem mass spectrometry (LC-MS/MS). Glyphosate, glufosinate and aminomethylphosphonic acid (AMPA) in blood and urine samples were analysed using Waters ACQUITY UPLC H-class system and Xevo TQ-S micro mass spectrometer. Sample preparation includes protein-precipitation with acetonitrile followed with centrifugation and filtration of the supernatant and lastly injecting the filtrate into the LC-MS/MS system. Chromatographic separation was achieved using a Waters Anionic Polar Pesticide analytical column. Mass spectrometric analysis was performed under electrospray ionization in negative-ion multiple reaction monitoring mode. Our optimized LC conditions successfully separated the target analytes, with acceptable linearities (R2 > 0.999), overall recovery ranged from 86% to 103%, and precision of less than 10% RSD. Thus, a working method with a simple and easy sample preparation procedure with no extra derivatization steps, and a short runtime of 12.5 mins, for simultaneous analysis of glyphosate, glufosinate, and AMPA in blood and urine samples were developed.

Keywords: Glyphosate, Glufosinate, AMPA, blood, urine

Introduction

Glyphosate and glufosinate are extensively utilized non-selective herbicides worldwide. Their widespread application has led to significant interest in understanding human exposure levels and potential health impacts. Aminomethylphosphonic acid (AMPA) is recognized as the primary and most stable metabolite of glyphosate, forming through oxidative deamination processes in both plants and soil [1]. Similarly, 3-methylphosphinicopropionic acid (MPPA) is identified as a major metabolite of glufosinate [2]. The presence of these compounds and their metabolites in human biological specimens is of considerable importance in exposure assessment, clinical toxicology, and forensic investigations, especially in cases of suspected poisoning [2].

The analysis of highly polar and ionic herbicide, the glyphosate, glufosinate, AMPA, and MPPA, in complex biological matrices, such as human blood and urine presents substantial analytical challenges. These difficulties arise from the unique physicochemical properties of these compounds, such as their high polarity, ionic nature, and absence of strong chromophores, which often necessitate intricate sample preparation and/or chemical derivatization to enable detection. Many traditional analytical techniques require derivatization to improve detectability, which increases sample preparation complexity and analysis time [3-7]. Recent advancements in liquid chromatography-tandem mass spectrometry (LC-MS/MS) provide an opportunity to directly analyze these compounds with high sensitivity and specificity without derivatization.

While several LC-MS/MS methods for glyphosate, glufosinate, and their metabolites have been reported [8, 9], many still involve complex sample extraction, such as solid-phase extraction (SPE) [8, 10], or samples are derivatised [2, 9, 10, 11, 12], or with tedious clean-up procedure [12], or are optimized for different matrices or applications [3, 4, 5, 13, 14, 15]. For instance, a method by Ohara et al. [11] involved a lengthy derivatization step for

for analysis, stating longer incubation results in more stable derivatized efficiency, while Guo et al. focused on a method that involves extraction using a modified Quick Polar Pesticides Extraction (QuPPe) method, cleanup with the PRiME HLB sorbents and detection with ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC–MS/MS) [16].

This preliminary study [17] presents the development of a high-performance LC-MS/MS method for quantifying glyphosate, glufosinate, and AMPA in urine and blood. Linearity, precision, accuracy, and recovery, was performed to ensure its suitability for routine toxicological analysis. Additionally, potential enhancements to improve method robustness are discussed. It is acknowledged that 3-methylphosphinicopropionic acid (MPPA) is a significant metabolite of glufosinate, often considered for comprehensive toxicological analysis. Due to its unavailability as a certified reference material at the time of this study, MPPA was not included in the current method development, but its inclusion will be a priority for future work.

Materials & Method

Materials

The urine samples used in this initial study were collected from a healthy volunteer. The blood was of bull's, collected from the local veterinary department. These samples were screened to ensure no interferences were observed at the retention time of the analytes of interest.

Certified reference materials of glyphosate, glufosinate, and AMPA together with a deuterated metabolite of glufosinate, D_3-3-methylphosphinicopropionic acid (D_3-MPPA) as internal standard were purchased from Sigma Aldrich (Switzerland). Waters Anionic Polar Pesticide, 5 µm, 2.1 mm x 100 mm column were provided by Waters Malaysia. Ultrapure water, chromatographic grades methanol and acetonitrile, and analytical grade formic acid (FA) were acquired commercially and used as received.

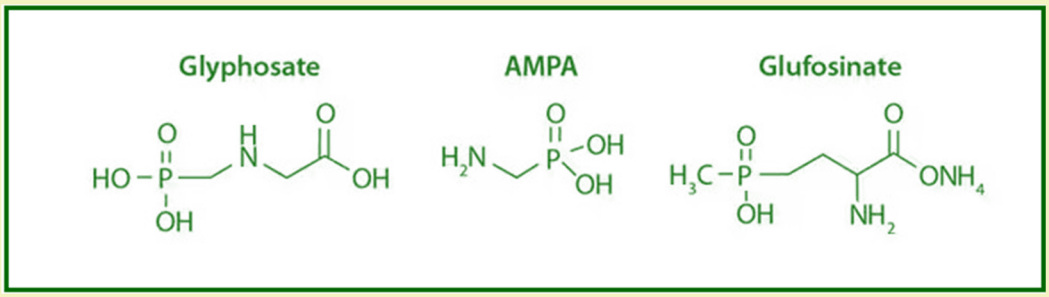


Figure 1: Molecular structures of glyphosate, glufosinate, aminomethylphosphonic acid (AMPA) and D_3-3-methylphosphinicopropionic acid (D_3-MPPA).

Sample Preparation

The sample preparation process was straightforward and efficient. The urine and blood samples were prepared through a simple extraction procedure. Two millilitres of acetonitrile were added to one millilitre of sample and vortexed, followed by spiking with d3-MPPA internal standard to a final concentration of 100 ng/mL. The mixture was chilled at -20°C for 90 minutes, centrifuged for five minutes, and the supernatant was filtered through a 0.22 µm syringe filter into polypropylene vials. Standards of glyphosate, glufosinate and AMPA were mixed to prepare 1 µg/mL in water. Calibration standards were prepared by serial dilution of stock solutions of glyphosate, glufosinate and AMPA in the respective matrices, at the concentrations of 10, 20, 50, 100, 200, and 500 ng/mL.

Instrumentation and Analytical Conditions

Chromatographic analyses were conducted using an ACQUITY UPLC H-class system equipped with a Waters Anionic Polar Pesticide column (2.1 × 100 mm, 5 µm). The flow rate was set to 0.5 mL/min, and an injection volume of 10 µL was used for all samples. The column temperature was maintained at 50°C, and the mobile phases

consisted of 0.9% formic acid in water (mobile phase A) and 0.9% formic acid in acetonitrile (mobile phase B). A gradient elution program was employed, starting with 90% A and 10% B, transitioning to 10% A and 90% B over 12.5 minutes.

Mass spectrometric detection was performed using a Xevo TQ-S micro triple quadrupole MS in electrospray ionization (ESI) negative mode. The capillary voltage was set at 1.5 kV, with a desolvation temperature of 350°C and a gas flow rate of 700 L/hr. The source temperature was maintained at 150°C. Multiple reaction monitoring (MRM) transitions were optimized for each analyte to ensure high specificity and sensitivity. Table 1 lists the MS conditions for all analytes; the first and second ions denotes the quantitative and qualitative transition product ions, respectively.

Table 1: The optimised MS conditions for Glyphosate, Glufosinate, AMPA and D_3-MPPA internal standard.

Glyphosate	168.28 > 62.94	38	20
Glufosinate	180.29 > 62.85	44	34
AMPA	110.35 > 62.85	48	16
d3-MPPA	154.35 > 62.88	36	28

Results and Discussion

The sample preparation by protein precipitation with chilling at -20°C for 90 minutes and filtration provided a good sample clean-up, and were acceptable for LC-MS/MS analysis. Figure 2 and Figure 3 presents the chromatograms of 10 ng/mL standards injection in urine and blood matrix, respectively.

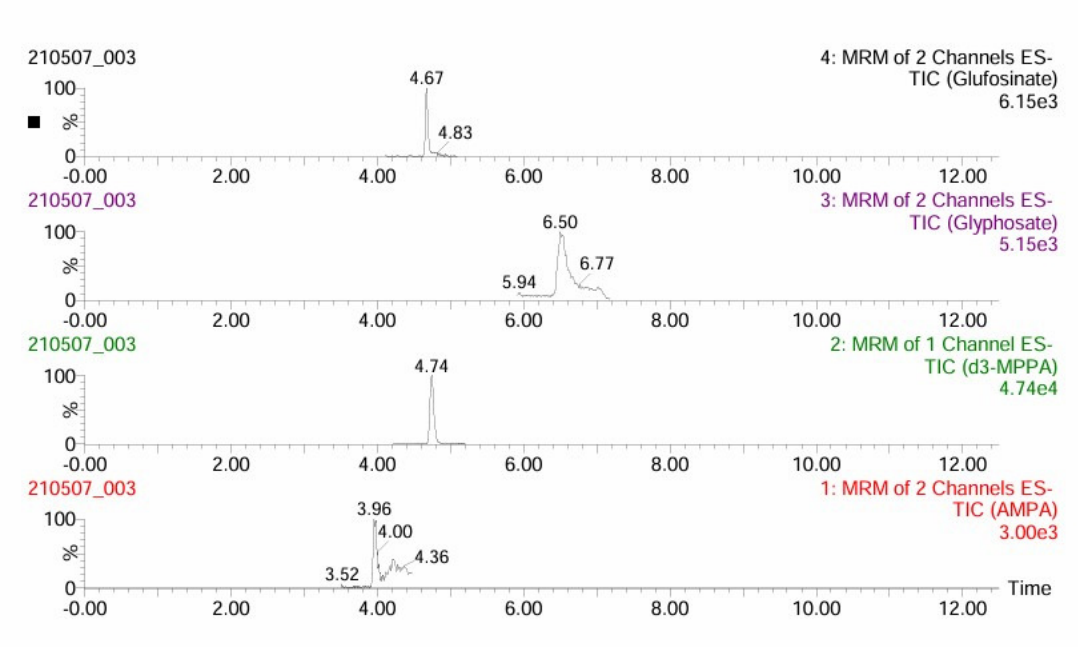


Figure 2: Chromatogram of 10 ng/mL standard mixture of glyphosate, glufosinate and AMPA in urine matrix. Retention time: glyphosate (6.50 min), glufosinate (4.67 min), AMPA (3.96 min).

Representative chromatograms of urine and blood samples spiked with glyphosate, glufosinate, and AMPA at 10 ng/mL demonstrated clear separation of the analytes. Retention times were consistent, with glyphosate at 6.46 minutes, glufosinate at 4.66 minutes and AMPA around 3.95 minutes.

A preliminary investigation was conducted to determine the feasibility of detecting target analytes at a concentration of 10 ng/mL, which was set as the limit of detection (LOD). The LODs of these analytes were determined by replicate analyses (n=7) of the urine and blood samples fortified at the lowest non-zero calibrator. The standard mixture was subjected to analysis, and both qualitative and quantitative detection were achieved, as shown in Figures 2 and 3.

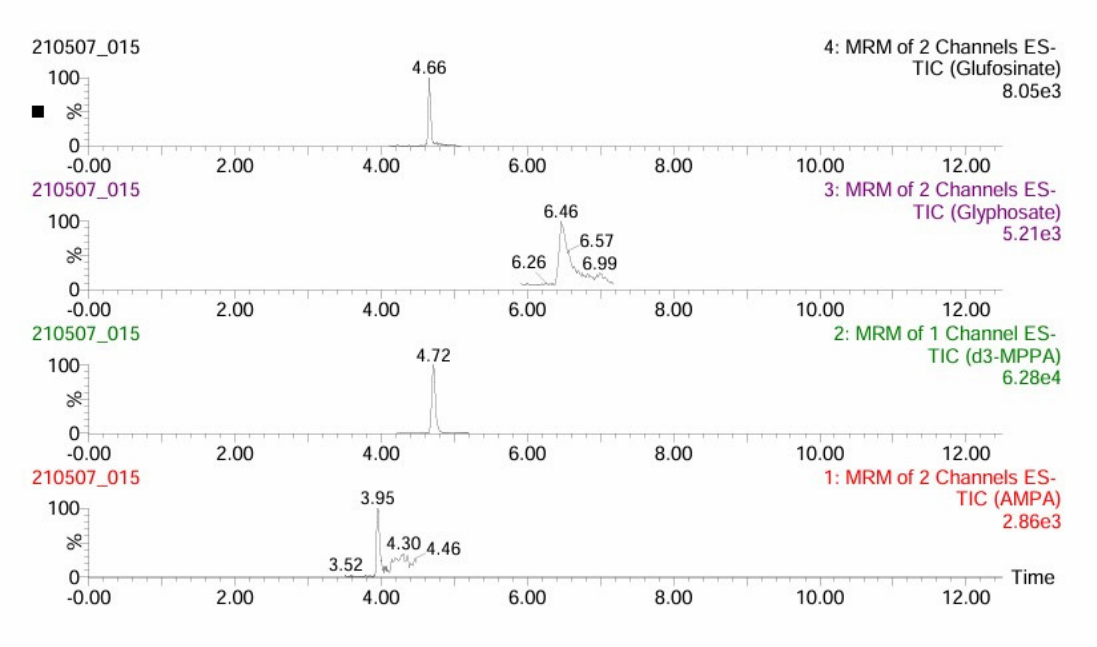


Figure 3: Chromatogram of 10 ng/mL standard mixture of glyphosate, glufosinate and AMPA in blood matrix. Retention time: glyphosate (6.46 min), glufosinate (4.66 min), AMPA (3.95 min).

The linearity, precision and recovery performance were evaluated. The method showed excellent linearity across the tested range (10–500 ng/mL), with correlation coefficients (R^2) consistently exceeding 0.999. This performance was consistent for all three compounds in both urine and blood matrices, as summarized in Table 2. However, only AMPA showed a slightly lower R^2 value in blood matrix.

Table 2: Linearity for glyphosate, glufosinate, and AMPA in urine and blood samples.

Glyphosate	10–500	0.9991	0.9998
AMPA	10–500	0.9996	0.9985

Precision and recovery results are as summarized in Table 3. The precision tests showed % RSD values below 10 % for all compounds, confirming the method's reproducibility. Recovery rates were high for glyphosate and glufosinate (86 % - 103 %) for urine and blood samples, while AMPA's recovery in blood was lower (~70 %), indicating room for improvement. These results are summarized in Table 2.

Table 3: Precision (% RSD) and recovery (%) for glyphosate, glufosinate and AMPA in urine and blood matrix.

Compound	Precision (% RSD)	Recovery (%)	Precision (% RSD)	Recovery (%)
Glufosinate	0.6	97 - 101	1.7	95 - 98

With a total of seven replicate analyses conducted on both urine and blood samples, the LC-MS/MS method developed in this preliminary study demonstrated exceptional analytical performance for the quantification of glyphosate, glufosinate, and AMPA in biological matrices. The method's high sensitivity and precision make it suitable for toxicological applications requiring the detection of trace levels of these analytes. The use of minimal sample preparation steps, such as protein precipitation with acetonitrile, simplifies the workflow, making the method amenable to high-throughput analysis.

One critical limitation observed was the reduced recovery of AMPA in blood samples, which was approximately 70 %. While matrix effects are commonly associated with such observations in complex biological samples, a formal assessment of matrix effects was not performed in this preliminary study. This omission prevents definitive conclusions regarding the exact cause of the reduced recovery. To address this limitation and thoroughly investigate the potential impact of matrix interferences, future work will include a comprehensive evaluation of matrix effects, potentially incorporating solid-phase extraction (SPE) techniques to improve both recovery and mitigate matrix interferences for AMPA.

Additionally, the use of a guard column could extend the lifespan of the analytical column, particularly when analyzing complex biological samples. This would reduce costs and improve method longevity in high-throughput settings.

The overall performance of the method, characterized by linearity ($R^2 > 0.999$), precision (% RSD < 10 %), and recovery (86 %-103 %), validates its robustness and reliability for routine analysis. The method's short runtime (12.5 minutes) further enhances its utility in high-throughput settings. While the method addresses most analytical challenges, ongoing improvements in sample preparation techniques and matrix interference management will enhance its applicability across diverse biological matrices.

To further confirm the robustness and reliability of the developed method for routine casework, several key validation parameters will be addressed in future studies. While linearity was assessed, the inclusion of standard residual plots will provide a more rigorous evaluation of the model's fit across the concentration range. Furthermore, a comprehensive investigation into matrix effects, process efficiency, and extraction recovery, as per established guidelines, will be conducted across a wider range of relevant biological matrices commonly encountered in casework (e.g., various antemortem and postmortem blood and urine samples). Crucially, the accuracy of quantitation will be systematically evaluated through the analysis of independent quality control (QC) samples prepared in these diverse matrices at different concentration levels to ensure the method's fitness for routine toxicological analysis.

Conclusion

This preliminary study successfully developed a rapid and sensitive LC-MS/MS method for the simultaneous quantification of glyphosate, glufosinate, and AMPA in human urine and blood samples. The method demonstrated good linearity, precision, and recovery (except for AMPA in blood), alongside a remarkably simple sample

preparation protocol and a short run time, showcasing its feasibility and potential for forensic toxicological applications. However, as a preliminary study, further comprehensive validation is required before its routine application in casework. Future efforts will focus on optimizing AMPA recovery in blood (e.g., through SPE), a formal assessment of matrix effects across diverse biological matrices (including antemortem and postmortem samples), and a thorough evaluation of accuracy using quality control samples. Additionally, the inclusion of other relevant metabolites, such as MPPA, will be considered to provide a more complete toxicological profile.

Acknowledgement

This study was supported by the Researcher-Industry Scientific Exchange (RISE) programme, an initiative of Ministry of Science Technology and Innovation (MOSTI). Under this programme, the Department of Chemistry Malaysia collaborated with Waters Analytical Instruments Sdn. Bhd. The authors gratefully acknowledge Waters Analytical Instruments Sdn. Bhd. for their technical assistance and data collection, especially to Dr KW Chong.

References:

1. *Kenny L, Sams C, Jones K, et al. Glyphosate – Determination of glyphosate and AMPA in urine by LC-MS/MS. Biomonitoring Method – Translation of the German version from 2025. MAK Collect Occup Health Saf. 2025;10(1): Doc021.*
2. *Yoshioka N, Asano M, Kuse A, et al. Rapid determination of glyphosate, glufosinate, bialaphos, and their major metabolites in serum by liquid chromatography-tandem mass spectrometry using hydrophilic interaction chromatography. J Chromatogr A. 2011;1218(23):3675-80.*
3. *Sancho JV, López FJ, Hernández F, et al. Rapid determination of glufosinate in environmental water samples using 9-fluorenylmethoxycarbonyl precolumn derivatization, large-volume injection and coupled-column liquid chromatography. J Chromatogr A. 1994;678:59-67.*
4. *Hori Y, Fujisawa M, Shimada K, et al. Quantitative determination of glufosinate in biological samples by liquid chromatography with ultraviolet detection after p-nitrobenzoyl derivatization. J Chromatogr B Analyt Technol Biomed Life Sci. 2002;767:255-62.*
5. *Kusters M, Gerhartz M. Enrichment and low-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in drinking water after cleanup by cation exchange resin. J Sep Sci. 2010;33:1139-46.*
6. *Bernal J, Bernal JL, Martin MT, et al. Development and validation of a liquid chromatography-fluorescence-mass spectrometry method to measure glyphosate and aminomethylphosphonic acid in rat plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2010;878:3290-6.*
7. *Motojyuku M, Saito T, Akieda K, et al. Determination of glyphosate, glyphosate metabolites, and glufosinate in human serum by gas chromatography–mass spectrometry. J Chromatogr B. 2008;875:509-14.*
8. *Li ZM, Kannan K. A method for the analysis of glyphosate, aminomethylphosphonic acid, and glufosinate in human urine using liquid chromatography-tandem mass spectrometry. Int J Environ Res Public Health. 2022;19(9):4966.*
9. *Hori Y, Fujisawa M, Shimada K, et al. Determination of the herbicide glyphosate and its metabolite in biological specimens by gas chromatography-mass spectrometry. A case of poisoning by Roundup® Herbicide. Journal of Analytical Toxicology, 2003;27(3):162–166.*
10. *Hori Y, Fujisawa M, Shimada K, et al. Determination of glufosinate ammonium and its metabolite, 3-methylphosphinicopropionic acid, in human serum by gas chromatography-mass spectrometry following mixed-mode solid-phase extraction and t-bdms derivatization. Journal of Analytical Toxicology. 2001;25(8)8:680–684.*
11. *Ohara T, Yoshimoto T, Natori Y, et al. A simple method for the determination of glyphosate, glufosinate and their metabolites in biological specimen by liquid chromatography/tandem mass spectrometry: an application for forensic toxicology. Nagoya J Med Sci. 2021;83(3):567-587.*
12. *Franke, A.A., Li, X. & Lai, J.F. Analysis of glyphosate, aminomethylphosphonic acid, and glufosinate from human urine by HRAM LC-MS. Anal Bioanal Chem. 2020;412: 8313–8324.*
13. *Narong C, Morris C, Harmon T. Direct Determination of Glyphosate, Glufosinate, and AMPA in milk by Liquid chromatography/tandem mass spectrometry. Journal of Regulatory Science, 2016;3(2):20-26.*
14. *Anastassiades M, Kolberg DI, Eichhorn E; et al. EU Reference Laboratory for pesticides requiring Single Residue Methods (EURL-SRM). Quick Method for the Analysis of Numerous Highly Polar Pesticides in Food Involving Extraction with Acidified Methanol and LC-MS/MS Measurement I. Food of Plant Origin (QuPPe-PO-Method). Version 11.1. 2021:84.*
15. *Schäfer AK, Vetter W, Anastassiades M. Improved Analysis of Glyphosate, Aminomethylphosphonic Acid, and Other Highly Polar Pesticides and Metabolites via the QuPPe Method by Employing Ethylenediaminetetraacetic Acid and IC-MS/MS. Journal of Agricultural and Food Chemistry 2025;73(4):2645-2652.*
16. *Guo H, Wang H, Zheng J, et al. Sensitive and rapid determination of glyphosate, glufosinate, bialaphos and metabolites by UPLC–MS/MS using a modified Quick Polar Pesticides Extraction method. Forensic Science International. 2018;283:111-117.*
17. *Waters Analytical Instruments Sdn. Bhd. Glyphosate and Metabolites Method Development (RISE05201036). 2021.*

A Review of Physical Evidence in Road Traffic Accidents in Sri Lanka

Mr Disanayaka Mudiyanselage Gayan Chanaka Bandara
Government Analyst's Department, Sri Lanka
Email: gayandc@yahoo.com

Introduction

The investigation of road traffic accidents (RTA) generally involves examining multiple factors, including the vehicles, their occupants, and any pedestrians involved, as well as the movements leading to the collision. Various reputed organisations have provided definitions in relation to road traffic accidents on different occasions [1][2], including the following:

"Accident" means an accident which occurs or originates on a road, street or any other place open to public traffic; which results in one or more person being killed or injured or causes damage to property; and in which at least one moving vehicle was involved, and includes collisions between vehicles, between vehicles and pedestrians and between vehicles and animals or fixed objects [3].

From the above, there are various types of RTA, and the factors involved in RTA may vary depending on the specific incident, region, time, and other circumstances. A systematic approach incorporating logical reasoning and problem-solving methodologies strengthens forensic investigation of RTA.

In Sri Lanka, RTA are normally reported to the nearest police station, where the traffic police officers will visit the scene, make preliminary evaluation about the accident and conduct a thorough investigation. Scene of Crime Officers (SOCO) may collect physical evidence from the accident site when required. If necessary, for example, when the driver does not accept responsibility, forensic experts from the Government Analyst's Department (GAD) may be engaged for further analysis.

Physical Evidence and Road Traffic Accidents

In the investigation of RTA, physical evidence plays a crucial role in determining the cause and responsibility of the accident. Physical evidence provides objective and scientific data that can be analysed to establish facts. It serves as a vital tool for investigators, enabling them to reconstruct the accident, identify contributing factors, and establish accountability.

In addition, in investigations, physical evidence is evaluated against eyewitness testimony to resolve ambiguities in the interpretation of the physical evidence [4].

In 2022, Sri Lanka recorded 2,395 fatal accidents, resulting in 2,515 deaths, including 792 pedestrians [5]. Some common types of physical evidence frequently encountered in RTA in Sri Lanka are given below:

1. Fibres and hair
2. Paint transfers and scratches
3. Broken vehicle parts (glass, mirrors, bumpers, etc.)
4. Vehicle damage and non-vehicular debris
5. Skid marks and tyre impressions

The evidence mentioned above is gathered by various authorities, including police officers, SOCO teams and forensic experts from the GAD. Subsequently, the evidence collected by police officers and SOCO teams are sent to the GAD, and samples collected by forensic experts from the GAD are directly brought back to the GAD for analysis.

Analysis of Physical Evidence Recovered in RTA

The GAD is the sole laboratory responsible for analysing forensic trace evidence in the country [6]. Physical evidence mentioned above is typically analysed in the Forensic Miscellaneous Laboratory (CM) at the GAD.

After receiving the evidence in relation to a road traffic accident, an initial assessment is carried out at the CM of the GAD to determine the type of analysis required based on the case background and the clarifications needed as indicated in the legal notice from the court.

The following sections discuss the analytical methodologies routinely employed by the GAD to analyse physical evidence frequently encountered in RTA.

Fibres and Hair

In hit-and-run accidents involving pedestrians, fibres are commonly collected as evidence. Police officers or forensic experts from the GAD identify and collect transferred fibres from the suspected vehicle, which are compared with the garments from the pedestrian.

Recently, the GAD has enhanced fibre analysis capabilities by introducing new equipment such as polarised light microscopes (as shown in Figure 1(a)) and Fourier Transform Infrared (FTIR) microscopes, as well as improving the existing fibre analysis procedures [6]. These advancements facilitate comprehensive cross-validation of evidence through multiple independent analytical methods, minimising procedural bias and error propagation.

With the introduction of new equipment and enhanced analytical procedures, fibre analysis can establish associations between the fibres recovered from the suspected vehicle and the control fibres from the pedestrian's garments more effectively and efficiently.

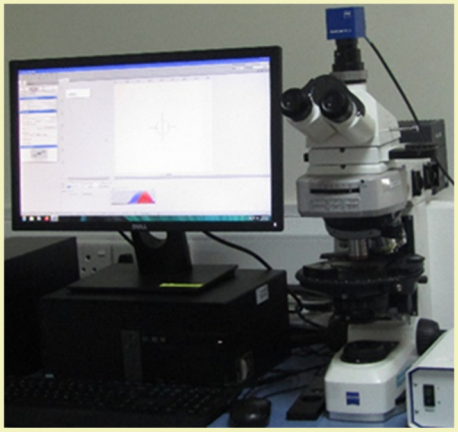


Figure 1: (a) A polarised light microscope at the GAD for analysing fibres.

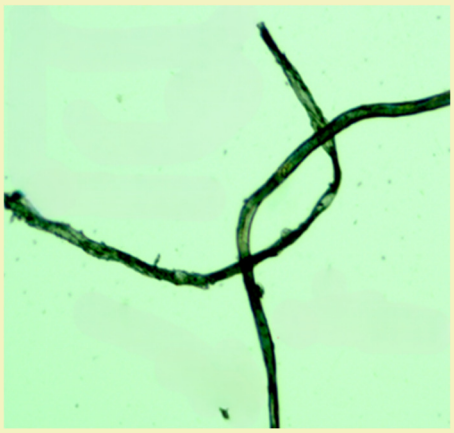


Figure 1: (b) A photomicrograph of fibres examined using the polarised light microscope.

Determining the causes of accidents can be challenging in cases without eyewitnesses, particularly for collisions involving animals. In some of these cases, forensic experts may be able to identify the animal species by analysing the characteristic features of the hair transferred to the vehicle during the collision.

Paint Transfers and Scratches

Paint transfers are valuable evidence in RTA, especially in vehicle-to-vehicle collisions. Control paint samples from the suspected vehicle's paint system and transferred paint found on the suspected vehicle are collected. During the investigation, transferred paint can be identified and subjected to further analysis.

A stereomicroscope (as shown in Figure 2(a)) is primarily used to locate and identify trace amounts of paint evidence for analysis. Detailed microscopic examination of paint fragments reveals their layer sequences, facilitating the identification of paint transfers between the vehicles involved in the collision.

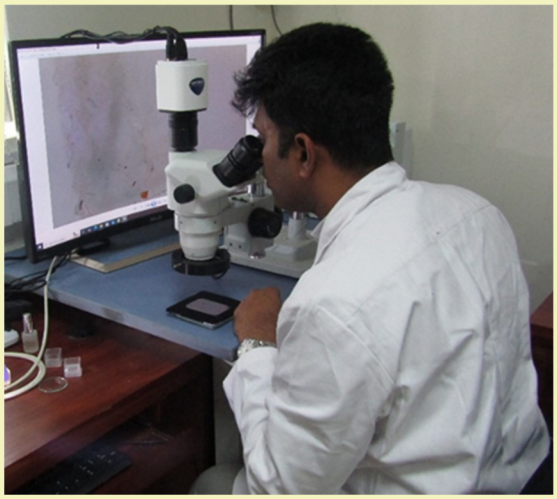


Figure 2: (a) A forensic expert using a stereomicroscope at the GAD to analyse physical evidence recovered from accidents.

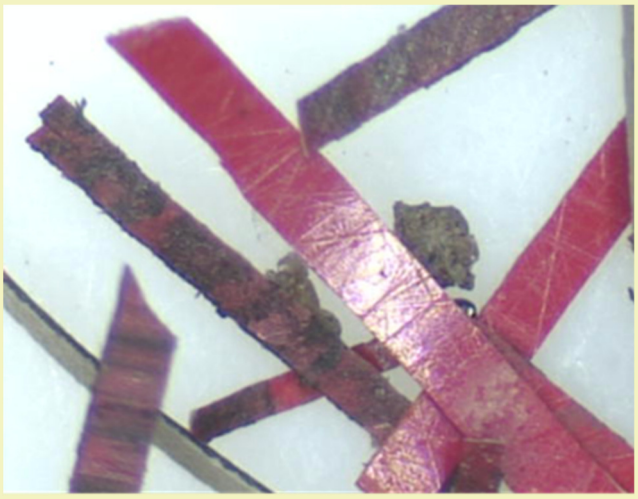


Figure 2: (b) A photomicrograph of a paint sample examined using the stereomicroscope.

Broken Vehicle Parts

After a collision, it is common to find broken vehicle parts scattered around the scene. This is especially prominent in RTA involving significant force between the colliding objects. While specialised instruments are often not required, establishing proper physical matches between broken vehicle parts requires thorough and careful observations. Physical fits between broken vehicle parts collected from the scene and the suspected vehicle can provide conclusive evidence of its involvement in the collision, and thus, have high evidential value.

Vehicle Damage and Non-Vehicular Debris

In some cases, drivers under the influence of alcohol or drugs may provide false information about the RTA, as admitting to impaired driving could affect their insurance claims or result in licence cancellation. Some drivers may attempt to conceal evidence of hit-and-run accidents, for example, by applying a fresh coat of paint to cover the damage. However, in many cases, they may fail to eliminate all traces of the incident.

When doubts arise about the accident, police or insurance investigators may require comprehensive forensic analysis. Unexpected physical evidence plays a critical role in verifying driver testimony, as it can either support or contradict their narrative on the incident circumstances. Environmental debris, such as cement plaster fragments and botanical matter from trees, may adhere to damaged vehicle surfaces. The nature of the vehicle damage can also indicate the type of object involved in the collision, particularly stationary objects such as posts and trees. Vehicle damage and debris can then provide corroborative evidence linking the vehicle to the specific location of the collision.

Skid Marks and Tyre Impressions

Vehicle tyres can leave impression marks on soft surfaces and skid or scuff marks on hard surfaces. Tyre marks play a crucial role in RTA scene investigations, even in cases where the vehicle involved is no longer present. When brakes are applied and the wheels lock, there is a higher chance of leaving skid marks on the surface. These marks help investigators determine the vehicle's direction of movement, tyre behavior, and speed at the time of braking.

Recent Development in the Analysis of Physical Evidence in RTA

In recent years, the investigation and analysis of physical evidence related to RTA in Sri Lanka have significantly improved with the adoption of new methods and technologies, which have enhanced traditional analyses and enabled new examination such as fibre identification and comparative material analysis. The enhanced expertise and efficiency in forensic investigations at the GAD have been possible due to international collaboration with relevant experts and in-service training programs. For example, in 2022 and 2023, the Korean International Cooperation Agency's (KOICA) project, in collaboration with the National Forensic Service (NFS) of Korea, provided valuable training on RTA analysis to forensic experts at the GAD.

Conclusion

Physical evidence in RTA investigations help provide a clearer and more detailed understanding of the incident. Such evidence supports various aspects of the investigation, including establishing a connection between the deceased pedestrian and the vehicle, identifying vehicles involved in hit-and-run accidents, determining the vehicle's association with the scene, verifying the consistency of statements given by drivers, and analysing the vehicle's movements at the time of the accident.

Hence, the proper identification, collection and analysis of physical evidence are essential in helping judges and juries resolve issues related to RTA accurately, thereby ensuring greater public confidence in judicial proceedings.

References:

1. *Parliament of the Democratic Socialist Republic of Sri Lanka. (2009). Motor Traffic (Amendment) Act, No. 8 of 2009. In Gazette of the Democratic Socialist Republic of Sri Lanka.*
2. *Road Traffic Act 1988, s170. Retrieved April 16, 2025, from https://www.legislation.gov.uk/ukpga/1988/52/section/170.*
3. *Road traffic accident. (n.d.). United Nations Office for Disaster Risk Reduction. Retrieved April 2, 2025, from https://www.undrr.org/understanding-disaster-risk/terminology/hips/tl0052.*
4. *The Royal Society & The Royal Society of Edinburgh. (2022). Forensic collision investigation: A primer for courts. https://royalsociety.org/-/media/about-us/programmes/science-and-law/des7309science-and-the-law-primercollision63101.pdf.*
5. *National Council for Road Safety. (n.d.). Retrieved April 2, 2025, from https://www.transport.gov.lk/web/index.phpoption=com_content&view=article&id=29&Itemid=149&lang=en.*
6. *Bandara, D. M. G. C. (2024). Recent Development and Trends in Trace Evidence Analysis in Sri Lanka. ForensicAsia, 14, 51–53.*

Case Study

Forensic Examination of a Modified Air Gun: Uncovering the Lethal Adaptation of a .22 Caliber Air Rifle in Sri Lanka

Mr. W.M. Ajith Kumara, Ms. M. Eranthi Jalika Seneviratne, Mr. W.A.R. Fernando, Mr. M.M.S. Premalal, Ms. I.H. Kulathunga, Ms. T.M.P.G.C.R. Seneviratne, Mr. C.D. Paranagama, Mr. P.D.G.S. Chathuranga*
Government Analyst's Department, Sri Lanka
**Email: ajithusjp@gmail.com*

Abstract

Illegal firearms remain a significant concern in Sri Lanka, despite strict firearm regulations. This case study documents the forensic analysis of a modified .22 caliber air gun, conducted by the Firearms and Ballistics Section of the Government Analyst's Department. The investigation revealed meticulous modifications, including a fabricated chamber and firing pin, making the air gun capable of discharging 5.56×45mm cartridges. The forensic examination showed that the tool marks on the spent cartridges recovered from the crime scene correspond with those produced by the modified weapon. This study demonstrates the conversion capabilities of air gun and underscores the necessity for stringent regulations on air guns.

Keywords: Modified Air Gun, Forensic Ballistics, Illegal firearms, Air gun adaptation, Forensic examination, Tool mark analysis, Sri Lanka.

Introduction

Air rifles have been popular among shooting enthusiasts, hunters, and sportsmen for decades due to their versatility, ease of use, and affordability. Unlike traditional firearms that rely on gunpowder, air rifles use compressed air, gas, or a spring mechanism to propel projectiles, usually small pellets or BBs. These rifles are commonly used for recreational shooting, target practice, pest control, and even competitive sports such as Olympic air rifle events. Over time, technological advancements have led to the development of more sophisticated air rifles with improved accuracy, power, and efficiency. One of the key reasons for the popularity of air rifles is their relatively lower noise levels and reduced recoil compared to conventional firearms, making them a suitable option for entry-level users in shooting disciplines and training environments. Additionally, in many countries, air rifles face fewer legal restrictions than conventional firearms, thereby offering greater accessibility. However, despite their lower power compared to traditional firearms, they still require responsible handling, as they can cause significant injury if misused. [1-3]

Firearms are strictly regulated in Sri Lanka under the Firearms Ordinance. However, the proliferation of illegally modified weapons [4] poses a growing threat to public safety. A notable amendment No 22 in 1996 to the Firearms Act excluded air guns from the definition of a "gun", enabling unrestricted ownership of .177 and .22 caliber air rifles [5]. This exclusion of air weapons from the firearm regulation has been exploited by individuals who obtain these weapons legally but subsequently modify them to fire live cartridges, in violation of the firearm laws.

The Firearms and Ballistic Section of the Government Analyst's Department plays a crucial role in examining such modified weapons. This paper presents a detailed case study of an illegally modified air gun, its forensic examination, and the legal implications of such modifications [6].

Case Study

Discovery and Seizure

A recent criminal case involved a .22 caliber air gun that had been modified to fire 5.56×45mm cartridges. Law enforcement authorities arrested a suspect in possession of the modified air gun, spent cartridges, and live cartridges and produced these items to the court. The court subsequently ordered the Firearms and Ballistic Section of the Government Analyst's Department to conduct a detailed forensic examination to determine the nature of the weapon and its use in criminal activities.

Received Court Case Items

In compliance with the court order, these items were received

1. An air gun
2. Seven (07) cartridges
3. Two (02) cartridge cases

Figure 1: Left-hand side of the air gun

Figure 2: Right-hand side of the air gun

Questions Submitted by the Court to the Firearms Examiner

The court submitted the following questions to the firearms examiner for evaluation:

1. Is the firearm an air gun, or has it been modified from an air gun?
2. Does this firearm meet the legal definition of a "gun" as stated in the Firearms Act?
3. Is this firearm classified as an automatic weapon?
4. What is the current operational condition of the firearm?
5. What is the status of the cartridges, are they live or spent cartridges?
6. Can the recovered cartridges be chambered in the firearm and is the firearm capable of firing them?
7. Can it be determined whether the spent cartridges were discharged from this firearm?

Figure 3: 5.56×45mm live cartridges

Figure 4: 5.56×45mm cartridge cases

Examination of the Air Gun

The forensic examination began with visual and mechanical inspection of the submitted air gun, compared with an exemplar air gun of the same make and model. The key modifications observed were:

1. Removal of the original barrel from the receiver. This was evidenced by crude welding marks at the barrel-receiver junction. The internal diameter of the new barrel measured approximately 5.60 mm, similar to the bullet diameter of a 5.56×45mm [7].

2. A customized chamber designed for 5.56×45mm cartridges was fabricated and welded to the receiver. The welding was irregular around the joint interface.

3. A firing pin was fabricated at the air release port, enabling primer strike upon trigger activation.

4. Reinforcement of the receiver structure with additional welding around the chamber area was observed. The weld seams showed variations in material texture and thickness when compared to an unmodified air gun.

The modifications exhibited precise engineering changes that required detailed inspection to identify. Borescope, Vernier caliper, Remote firing system, and Comparison Microscope (Projectina) were used for these examinations.

Figure 5: Customized chamber to fit 5.56×45mm cartridge

Figure 6: Fabricated firing pin at the air release port

Ballistic Testing and Tool Mark Analysis

To assess the operational capability of the weapon, test firings were conducted using the remote firing system. The test firings were conducted using 5.56×45mm cartridges with headstamps similar to those recovered at the crime scene. The modified air gun successfully discharged all test cartridges, and these test-fired cartridge cases were collected for examination.

A comparative analysis was performed between the test-fired cartridge cases and the recovered cartridge cases. Microscopic examination revealed the firing pin impressions on the recovered cartridge cases displayed corresponding individual characteristics with those produced by the modified air gun. This agreement in class and individual characteristics on the firing pin impressions indicate that the recovered cartridge cases were fired from the submitted modified air gun.

.

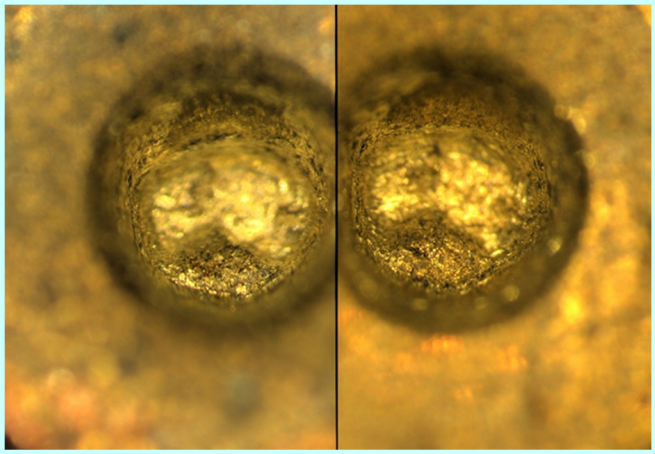


Figure 7: Microscopic comparison of firing pin impressions on recovered cartridge cases from the crime scene.

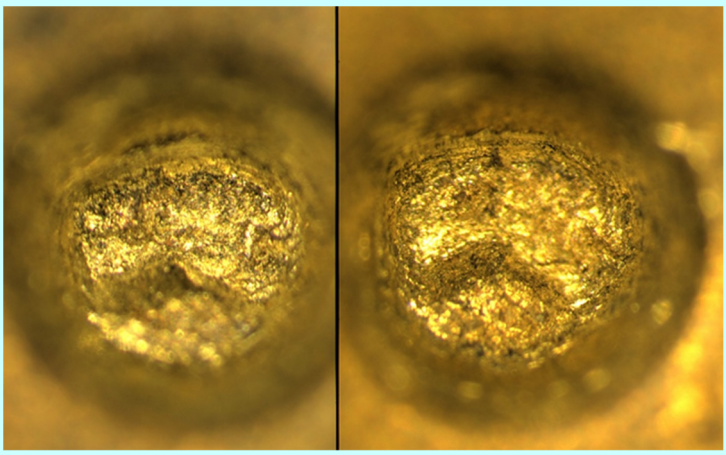


Figure 8: Microscopic comparison of firing pin impressions — recovered cartridge case (left) and test-fired cartridge case (right).

Legal and Forensic Findings

Based on the forensic findings, the following conclusions were drawn:

1. The examined item was originally an air gun that had been modified to discharge 5.56×45mm cartridges.
2. Under Section 2(3) of the Firearms Ordinance of Sri Lankan, the submitted air gun, following conversion to discharge center-fire ammunition, meets the definition of a "gun". Possession of such a converted weapon without a license constitutes an offense under Sri Lankan law.

.

3. The weapon does not meet the technical criteria for classification as an automatic firearm under Section 2(1) of the Act.

4. The submitted air gun was operational and successfully discharged all the test cartridges when activated.

5. The seven (07) recovered cartridges were identified as live 5.56×45mm cartridges based on visual and physical examination of their intact primers and projectiles. The two (02) recovered cartridge cases were identified as spent 5.56×45mm cartridges based on the presence of firing pin impressions and absence of intact primers and projectiles.

6. The submitted air gun demonstrated capability to chamber and discharge 5.56 × 45mm cartridges.

7. The firing pin impressions on the recovered cartridge cases correspond to those on the test-fired cartridges, indicating the recovered cartridge cases were discharged from the submitted air gun.

Conclusion

The forensic examination revealed that modification of the submitted air gun enabled it to discharge 5.56x45mm cartridges. The modification included fabrication of the chamber and a firing pin. In addition, the internal barrel diameter of the submitted .22 caliber air gun corresponds sufficiently to the bullet diameter of 5.56×45mm cartridges, facilitating such modification as there is no need to alter the barrel dimension.

The Firearms and Ballistic Section in the Government Analyst's Department identified the modifications of the submitted air gun and established the linkage between the recovered cartridge cases and the seized weapon. These findings contribute to the understanding of air gun modifications and highlight the importance of advancing forensic techniques in firearm analysis. The findings of this case underscores the possible risk of modifying legally available air guns into functional firearms, contributing to discussions on air gun regulation policies in Sri Lanka.

References:

1. *Gunethilake KMTB, Vidanapathirana M. Medico-legal issues in air gun injuries. IOSR J Dent Med Sci. 2016 Aug;15(8 Suppl 10):146–8. Available from: https://www.researchgate.net/publication/307535729_Medico-Legal_Issues_in_Air_Gun_Injuries.*
2. *FrontPage. Rifle misfires killing five-year-old child [Internet]. FrontPage.lk. 2022 Nov 17 [cited 2025 Mar 11]. Available from: https://frontpage.lk/rifle-misfires-killing-five-year-old-child/.*
3. *Siddiquea MS, Vadysinghe AN, Ekanayake KB, Fernando DMG. Near-fatal air gun injury in a child – case report. Sri Lanka J Med [Internet]. 2022 Aug [cited 2025 Mar 19];31(2):46–8. Available from: https://doaj.org/article/03ff7814a68c4b7f86af8f3d2c813e84.*
4. *Associated Press. Deadly Thai mall shooting exposes murky trade in blank handguns that are turned into lethal weapons [Internet]. AP News. 2023 Oct 5 [cited 2025 Apr 11]. Available from: https://apnews.com/article/blank-handguns-gun-control-lethal-weapons-038d4dc5b9a16790471d06e6aa1436b9.*
5. *Firearms (Amendment) Act, Sri Lanka, No 22 of 1996.*
6. *Mendis MAJ. Roadmap to Criminal Investigations [Internet]. Harindranath Chandrasiri; 2013 [cited 2025 Mar 19]. Available from: https://books.google.lk/books?id=IXiYnQAACAAJ. ISBN: 9789559522522.Mendis M A J, Roadmap to Criminal Investigation, 2023.*
7. *Anthony G Williams, Jayesh Dhingra, HIS Jane's Weapons Ammunition book, United Kingdom, Hobbs Printer, 2017-2018.*

Case Study

Types of Forensic Linguistic Examinations in the Judiciary of Mongolia

Khulan Ganbold
Questioned Document Laboratory, National Forensic Agency of Mongolia, Police Major
Department of Mongolian Language and Linguistics, National University of Mongolia, Mongolia
Email: ganboldkhulan61@gmail.com

Crime has been a persistent phenomenon in human society, and as society evolves and transforms, the types, methods, and forms of criminal activity continue to grow increasingly sophisticated.

As a result, the need to utilize specialized scientific knowledge in the process of criminal investigation and evidence gathering has steadily increased. Within this context, it has become a pressing priority to define the theoretical foundations of forensic linguistics, particularly linguistic forensic examination, to classify the various types of such examinations, and to further refine the methodologies and techniques applied in each type of analysis.

Keywords: Forensic examination, Forensic science, Forensic linguistics, Linguistic forensic examination, Types of examination

Introduction

Globally, forensic science has been recognized as an independent field of knowledge since the 20th century. In Mongolia today, it has expanded into various specialized branches, including forensic criminalistics, forensic natural sciences, forensic medicine, forensic engineering and technical examination, forensic digital and cyber examination, and forensic economic examination. Similarly, linguistic forensic examination is gradually emerging, and it is only a matter of time before it develops into a fully independent branch of forensic science.

The concept of 'forensic linguistics' is a relatively young discipline compared to other branches of forensic examination, having emerged and developed since 1968. Its application varies across countries depending on their respective legal systems. In the case of Mongolia, this field has not yet developed into a specific discipline, and its theoretical environment, legal foundations, and organizational structure are still underdeveloped and not clearly defined.

Forensic linguistics aims to interpret and identify the unusual language and writing used by offenders, to facilitate the swift detection of crimes, and to contribute to the collection of forensic evidence. Therefore, a forensic linguist must possess broad and in-depth knowledge across various linguistic subfields, including phonetics, morphology, syntax, semantics, discourse analysis, pragmatics, psycholinguistics, neurolinguistics, sociolinguistics, computational linguistics, and corpus linguistics. Forensic linguistics directly interacts with and applies methodologies from related disciplines such as sociolinguistics, corpus linguistics, psychology, mathematical linguistics, text linguistics, and computational linguistics. *(Danielewicz-Betz, 2012:94; Hazhar Ramadhan Ahmed, 2021:23)*

Forensic linguistics (also referred to as legal language) is a subfield of applied linguistics that analyzes linguistic traces—such as legal language, documents, and written or spoken texts related to criminal acts—in order to use them as evidence in criminal investigations. Based on such definitions (Munkhtsetseg & Munkh-Uchral, 2022), the fundamental concept of forensic linguistics can be understood.

Forensic linguistics is an interdisciplinary field situated between forensic science within the legal domain and sociolinguistics. In other words, the theoretical foundations, subject matter, research methods, legal framework, and organizational aspects of forensic linguistics must be examined from both legal and linguistic perspectives in an integrated manner.

Clarifying the types of linguistic forensic examinations holds significant practical value and is essential to the theoretical framework of

forensic linguistics. It also establishes a foundation for the development of expert training programs and the formalization of examination procedures. Therefore, this article aims to identify the necessity and demand for linguistic forensic examinations in Mongolia and to classify the various types of such examinations.

Core Section

The International Association of Forensic Linguists (IAFL) was established in 1992 by forensic linguist Malcolm Coulthard and his colleagues (Li He Yan, 2020). The Association classifies the field of forensic linguistics into four main areas of focus: (1) *Language and Law*—concerned with the clarity, wording, and interpretation of legal texts to avoid ambiguity in legal documents and legislative drafting; (2) *Language in the Process*—focused on analyzing language used in legal proceedings, including interactions between participants, witness statements, and interpreting or translation issues; (3) *Language as Evidence*—which involves identifying, attributing, or interpreting spoken or written texts relevant to a case, including authorship attribution and interpretation of meaning in legal disputes; and (4) *Research and Teaching*—which involves training forensic linguists and conducting academic research in the field (iafl.org/forensic-linguistic/). Among these, the categories of *Language in the Process* and *Language as Evidence* fall directly within the scope of forensic linguistic examination.

Various types of forensic linguistic analyses are discussed in academic literature, including author identification, forensic stylistics (semantic and pragmatic meaning analysis), discourse analysis, forensic phonetics, textual analysis, and authorship attribution of SMS messages (Danielewicz-Betz, 2012: 95–98). Additionally, other areas such as dialectology (linguistic dialect analysis) and forensic transcription are covered in works by Ariani, Fatemeh & Mahin (2014: 223). In the Russian Federation, author identification through comparative linguistic analysis is conducted using the refined methodology developed by T.V. Averyanova (2003). Furthermore, forensic institutions also perform analysis to determine the presence of offensive or defamatory language, or expressions violating ethical norms, as outlined by Izotova et al. (2016: 93).

In international practice, the types of forensic linguistic examinations are categorized as described above. However, in our country, although there is no official document issued by researchers that defines and classifies the types of forensic linguistic examinations, some works by forensic science scholars have touched upon the theories and methodologies related to author identification—one of the subtypes of forensic linguistic analysis (Khurtsgerel, 2002: 437–441; Jargalsaikhan, 1998: 100–102; Bat-Erdene, 2014: 81–84). In these works, the methodology for identifying the author of a written text is considered a sub-discipline of forensic document examination, specifically referred to as "forensic author identification." It is stated that the direct object of this type of analysis is the written language, which reflects both general and individual linguistic habits (Khurtsgerel, 2002: 438). Furthermore, a written text (letter) is seen as a medium for transmitting information over distance or as an auxiliary tool of human communication. It is also posited that any written text contains the following two fundamental characteristics:

- Textual meaning (defines the content of the document)
- Textual technique (handwriting style)

The semantic aspect of the text reflects the linguistic knowledge and writing composition skills of the executing party. The term *author of the text* refers to the person who produced the text, though the author and the executor may be different individuals. The author of a text is identified based on distinctive linguistic features, and this process is called author identification analysis (Jargalsaikhan, 1998: 100). The uniqueness of the conditions under which an individual is formed leads every person to acquire specific, inimitable norms of expression and thinking based on their linguistic and cognitive habits. These very characteristics form the basis for identifying the author of the original text. Personal linguistic features include: Orthographic rules (spelling), punctuation usage, and the unique application of punctuation marks, Division of the original text into paragraphs and lines, Lexical and syntactic habit markers (vocabulary and sentence structure), Stylistic and compositional language features (Khurtsgerel, 2002: 440). Although a methodology for conducting author identification analysis through the processing of linguistic markers does exist, only one such analysis was conducted in 2015, and it appears that this type of analysis is rarely performed. The reason why this type of examination is not being conducted lies in the fact that forensic experts in other branches of criminalistics and forensic science lack specialized linguistic knowledge. Furthermore, the officials authorized to assign forensic examinations during the investigation and adjudication processes have insufficient understanding of forensic linguistic analysis.

In order to examine the practice of conducting this type of analysis, a review of expert conclusions

stored in the archives of the Forensic Science Institute reveals that a total of five forensic linguistic analyses have been conducted: three in 2012, and one each in 2015 and 2023. These documented cases fall under the scope of forensic linguistic examination. When classified according to the object of examination, they include:

- Five printed texts concerning specific individuals running for election;
- A two-page printed text addressed to a specific political figure;
- One mobile phone (containing stored messages and information);
- One text written in a notebook.

A review of the questions posed by authorized officials when requesting expert opinions related to forensic linguistic analysis reveals the following inquiries:

- In four of the examinations: "Were the printed texts submitted for analysis written by the same person?"
- In two examinations: "What is the meaning and content of the letter submitted for analysis?"
- In two examinations: "Is it possible to determine the education level or profession of the person who wrote the submitted printed texts?"
- In one examination: "By comparing the writing style, font selection, and format of the messages sent from the specified number on the mobile phone, is it possible to determine whether they match the writing of the named individual?"
- Additional questions included: "Which field or domain do the terms used in the text likely belong to?" and "Could the language used be characteristic of gamblers?"

An analysis of the expert conclusions in response to the questions listed above reveals the following outcomes:

- One case concluded: "It is not possible to determine whether the texts were written by the same individual."
- Two cases concluded: "It is possible that the texts were written by the same person," and one case stated: "It is also possible that one of the letters was written by a different individual who copied the content or idea from the other letter."
- One case concluded: "The texts may have been written by different individuals."
- One case stated: "It is not possible to determine the education, profession, or social background of the author."
- One case stated: "Based on the content, structure, and composition of the letter, it is likely that the author possesses a high level of writing proficiency."
- One case included a determination of the meaning and content of the text.
- One case stated that it was not possible to determine the meaning and content of the text.
- One case concluded that the language used in the text may be characteristic of terminology used by gambling (card game) players.

From these findings, it can be observed that the objects of forensic linguistic analysis are primarily documents such as letters, notices, and handwritten notes containing printed texts related to political matters or involving issues such as defamation, insult, or threats against public figures. In terms of types of analysis, they include author attribution, author identification, and determination of the meaning and content of the text. Since 2013, the forensic science institution has been conducting phonetic forensic analysis, with an average of 15 audio recordings and 100 video recordings examined annually. Among these, approximately 50% of audio analyses and about 30% of video recordings with speech involve questions related to forensic linguistic analysis. These inquiries typically include:

- Determining the sequence of spoken words;
- Establishing the meaning and content of speech;
- Identifying the tone of speech (e.g., angry, crying, threatening, fearful, shocked);
- Clarifying specific words or phrases in the speech;
- Determining the regional dialect or origin of the speaker.

However, due to the lack of specialized linguistic knowledge among digital and electronic technology experts, these questions cannot be adequately addressed. Therefore, it is evident that there is a high demand and necessity for conducting forensic linguistic analysis on speech found in audio and video recordings.

Furthermore, interviews with linguistics professors and researchers from universities, as well as linguistic experts from the Academy of Sciences, revealed that they do perform forensic linguistic analysis and provide conclusions regarding spoken and written materials related to legal disputes. However, several challenges were noted: such work does not fall within their official duties, they are not compensated for conducting these analyses, and they are often summoned to court as witnesses. The types and

number of analyses conducted by linguistic experts remain unclear, which is related to the fact that forensic linguistic conclusions are not prepared in accordance with a standardized methodology developed by forensic science. Additionally, the expert conclusions are not archived or preserved in an official manner.

Thus, the fact that linguistic experts are conducting forensic linguistic analyses constitutes a form of analysis performed outside official forensic institutions. Although such activities were not specifically regulated prior to 2023, the revised version of the Law on Forensic Examination, which came into effect on January 1, 2023, formally legalized this practice by introducing a provision allowing for "examinations to be conducted by other authorized entities." This legal provision now governs and regulates the conduct of forensic analyses outside the official forensic examination institutions.

Therefore, in accordance with the above-mentioned legal provision, linguistic experts are now able to officially conduct forensic linguistic examinations by obtaining authorization from the forensic science institution, thereby creating the legal framework and opportunity for them to perform such analyses in an official capacity.

Based on the above, forensic linguistic analysis may be classified and categorized into the following types:

- Author attribution analysis – determining whether various texts (such as messages stored on mobile phones, letters, online posts, comments, emails, printed or handwritten texts) were written by the same individual; establishing whether a printed text was written by a particular person by comparing it with other texts written by that individual.
- Author profiling analysis – identifying characteristics of the author such as ethnic or regional background, profession, and level of education.
- Semantic and content analysis – determining the specific field or domain of terminology used; identifying whether the text contains threatening, insulting, coercive, or inciting content; and clarifying the meaning and intent conveyed by spoken or written language.
- Phonetic analysis – clarifying unintelligible words; identifying emotional tones such as fear, shock, anger, or happiness based on speech patterns.

Furthermore, linguistics experts must obtain authorization to conduct forensic examinations in accordance with the grounds and procedures stipulated by law. Within the scope of their specialized knowledge, they are required to carry out in-depth studies on various types of linguistic forensic examinations, develop methodologies for such analyses, and, based on these, conduct examinations in accordance with scientifically established forensic methods and procedures. The conclusions derived from these examinations must be capable of being evaluated as sources of evidence within criminal investigations and judicial proceedings. In addition, conducting broader studies on issues related to forensic linguistics - including theoretical, legal, and organizational aspects - is considered essential for laying the foundation for the development of this field.

References:

1. *Ahmed, H. R. (2021). The role of forensic linguistics in crime investigation: uses in legal proceedings. Kurdistan Region, Iraq. Anglisticum Journal (IJLLIS), 10(2,3), 23.*
2. *Coulthard, M. (2010). Forensic Linguistics: the application of language description in legal contexts. London.*
3. *Coulthard, M., & Johnson, A. (2014). An introduction to forensic linguistics: language in evidence. UK.*
4. *Danielewicz-Betz, A. (2012) The role of forensic linguistics in crime investigation. In Language Studies: Stretching the Boundaries by Littlejohn, A. and Rao, M. S. (Eds): Cambridge Scholars Publishing*
5. *Mohsen Ghasemi Ariani., Fatemeh Sajedi., Mahin Sajedil. (2014). 14th International Language, Literature and Stylistics Symposium, Forensic linguistics: A brief overview of the key elements. Procedia-Social and Behavioral Sciences 185 (2014) 222-225.*
6. *Averyanova, T. V. (2003). Criminalistics. Moscow, Russia: Norma-Infra.*
7. *Bat-Erdene, B. (2017). Criminalistics. Ulaanbaatar, Mongolia: Arvin Sudar Company.*
8. *Jargalsaikhan, B. (1998). Criminalistic Techniques. Ulaanbaatar, Mongolia: Printing Laboratory of the Defense University.*
9. *Izotova, T. M., Kuznetsov, V. O., & Plotnikova, A. M. (2016). Methodology for Conducting Forensic Linguistic Examinations in Cases of Insult. Theory and Practice of Forensic Examination, 1(41), 92–97.*
10. *Lee Hae Yang. (2020). Forensic Linguistics. Seoul, South Korea: Jungang Publishing.*
11. *Lkhagvasuren, B. (2017). Human Rights-Based Forensic Examination: Theoretical and Methodological Issues. Ulaanbaatar. Doctoral dissertation in Law.*
12. *Munkhtsetseg, N., & Munkh-Uchral, E. (2022). Mongolian Linguistics: Applied Linguistics, Language Processing, and Universal Grammar. Ulaanbaatar, Mongolia: Institute of Mongolian Studies, National University of Mongolia.*
13. *Ochirbat, T. (2024). Foundations of Forensics. Ulaanbaatar.*
14. *Khurtsgerel, J. (2002). Criminalistics I. Ulaanbaatar, Mongolia: Ulaanbaatar Times Newspaper Printing House.*

Case Study

Utilization of Insects to Analyze the Minimum Post Mortem Interval ($_{min}$ PMI)

Miss Prapaipit Buadilok
Central Institute of Forensic Science, Thailand

Abstract

During death investigations, insects are mostly used to estimate the Post Mortem Interval (PMI) and useful for the forensic science. Especially getting entomologists who have knowledge and work experience. These estimates are only as good as they are close to the true PMI (Sharma Ruchi, Rakesh Kumar Garg and J. R. Gaur, 2015). By studying the life cycle, behavior and habitat of insects. The use of forensic entomology is one of the fastest growing methods used to determine time since death under law enforcement Forensic Science Act (Thai Forensic Act, 2016). The objective of this paper is to analyze and evaluate how forensic entomology is beneficial and used to estimate the period of time. The experiment explores and examines the eggs and maggots that are found on a body at the crime scene and then sent to the autopsy room, Egg and Maggot samples were collected from the corpse for testing by sectioning the posterior spiracle area of the maggot and then it was taken and looked at under a microscope. From the study, the second and third stage blow fly larvae were found, and it was estimated that this body had been dead for at least 20 - 30 days, which was a time since the death of the Post Mortem Interval (PMI) with the process, (Aballay, Fernando H., M. Cecilia Dominguez, and Florencia Fernandez Campón, 2012) also the decomposition of corpses too (Payne, 1965). This is very useful for entomologists in estimating mortality as short as possible and as close as possible to the postmortem interval. Forensic Entomology minimum Post Mortem Interval ($_{min}$ PMI). (Matuszewski, 2021)

Keywords : Forensic science, Forensic entomology, Maggots.

Introduction

Forensic Entomology was first used in 1235 in China by Sung Tz'u, who was a “Death Investigator” and he wrote a book in the Medico-Legal Textbook titled “Hsi Yüan Chi Lu” in Chinese. English as "The Washing Away of Wrongs". This is the first book written for investigative purposes (Dorothy, 2007). In the book, Mr. Sung also educated about the blow fly that likes to swarm on the open organs of the corpse, such as the eyes, ears, the nose, mouth, anus and vagina, especially wounds. In the 19th and early 20th centuries, forensic entomology began to be used in more investigations. Until in 1996, the American Board of Forensic Entomology was established, which is a certification board for forensic entomologists (ABFE, 1996) in Thailand. There is the first report on forensic entomology by a professor from Chiang Mai University. The body was found near the police station in Lampang province. On the corpse's leg, a honeycomb-like wound was found, with a large number of larvae in the wound. The maggots were sent to the medical team in Department of Parasitology of Chiang Mai University, and studies found the maggots in stage 3. Its scientific name is *Chrysomya rufifacies* (Macquart). From this information, it can be used to estimate the time of death (Sukontason, Kabkaew L., K. Sukontason, P. Narongchai, S. Lertthamnongtham, S. Piangjai and J. K. Olson, 2001)

The use of entomologists in crime scene investigations is becoming increasingly important as insects arrive at the scene quickly and are considered excellent first evidence and first witness (UiTM, 2014). Today, the use of insect and animal evidence to determine time since death is accepted in modern forensics. However, entomologists still have to study, research, find the new knowledge to apply for work, maximize the benefit of solving cases and providing justice.

Case Study

In this research, it is Case A2089/51, Bang Pa-In Police Station, Phra Nakhon Si Ayutthaya Province. The found body was decayed and withered, leaving only the skin and bones, the hair falling off, and the skin turned brown black. Eggs and maggots were

found on the bodies in Figure 1, Figure 2 , and Figure 3 respectively. In general, flies lay eggs, taking about 1-2 days to hatch from maggots, depending on many factors such as temperature (Deonier CC, 1940), light, humidity, etc. These factors are all interrelated. is especially important in assessing the Post Mortem Interval (PMI) (Aballay, Fernando H., M. Cecilia Dominguez, and Florencia Fernandez Campón, 2012).

This research was done by a professional entomologist who worked and collected insects and arthropods more than 19 years to make a low mistake in estimating the least and most realistic PMI or $_{min}$ PMI (minimum Post Mortem Interval).

Objective: To estimate the time after death as accurately as possible by studying the behavior, life cycle and habitat of flies coupled with the decomposition process of the remains (Davies, 2000).

Materials and Methods

Take a sample from the corpse. These are creamy-white maggots found on the face, neck, chest, body, arms, and legs. In collecting it have to be recorded as follows:

1. Details of the terrain: city, countryside, desert, forest, grassland, etc.
2. The place where the body was found: the ground condition is sandy soil, loamy soil, clay rock, hillside, plain, whether there is a road through it, etc. Type of plant found What kind of plant is it? Aquatic plants, endemic plants, etc. If you don't know (ask a botanist).
3. Date, time, and temperature
4. Species of insects (arthropods) found
5. Case number
6. Recorder's name (Rowe, 2007)

Note: In taking notes on the collection of samples from the crime scene, photography is also an important part of helping researchers work more efficiently (Lee and W.D. Lord, 1989).

Steps to Make Slides:

1. Take the insects (specimens) collected from the corpse and then bring them to the cutting laboratory and place it in the cassette. Bring the cassette to the automatic tissue processor for the preparation process, treated with a chemical solution so that the treated specimen is sufficiently hardened to be sectioned (Adams and Hall M. J. R. 2003).

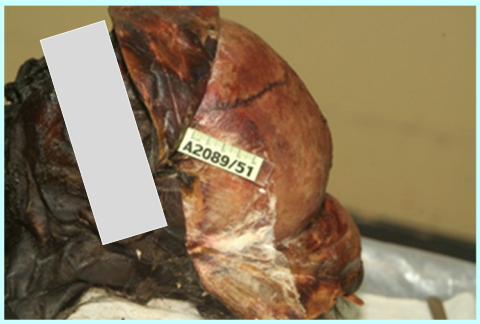


Figure 1: Eggs

Figure 2: Maggots

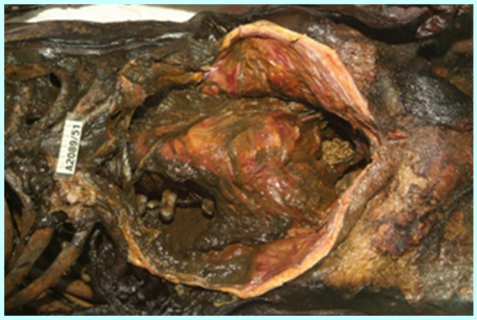


Figure 3: Maggots

2. Prepare the chemically specimen through an automatic tissue processor and embed it in a liquid wax medium contained in a mold of various sizes according to the size of the specimen using a tissue embedding machine. The hardening medium will support the specimen in such a way that it can be easily cut into thin tissues 3-5 microns in size by following (Making Permanent Microscope Slides of Insects) and also recording the number of blocks in the embedding record. Insert the cartridge with the Tissue Embedding center.

3. Take the embedded specimen into the paraffin block and cut it into thin tissue (paraffin section) with a Rotary microtome. Normally, we will cut the thin flat tissue, 3-5 microns in size, with the tissue plate embedded on a glass slide. By following, specimens should be cleared to allow transmitted light to pass through them. Clearing dissolves soft body parts, allowing the structure of the cuticle to be seen in its entirety once the specimen is slide mounted. There are two methods for clearing:

3.1) Cold Potassium Hydroxide (KOH) - Leave specimens in 10% KOH solution at room temperature overnight. After clearing with KOH, specimens should be returned to pH neutral water or alcohol before being passed through the alcohol series for dehydration.

3.2) Warm KOH - Specimens in 10% KOH can be heated on a double burner over an electric hot plate. This will simply speed up the clearing process. You should be careful with this technique becasue it often clears specimens too quickly and KOH sputters strongly when heated. After clearing, specimens should be returned to pH neutral water before being passed through the alcohol series. (Nelson Riley C and Karin Gastreich, 2001).

4. Put the glass slides with paraffin section into the hot air oven by adjusting the temperature into two ranges, namely 90-110 °C for 15-20 minutes and 60-65 °C for 30-60 minutes to help. The adhesion of the paraffin section to the slide surface prevents the section from falling off easily during staining. Staining: Two types of cuticle stains may be used: acid-fuschin and Harris hematoxylin. These can be added to your specimens while they are in 70% alcohol. The specimen will become darker and darker as time in the stain increases. Some of the stain will be leached from the specimen in later stages of the dehydration series so tests should be run to determine the level of "overstaining" necessary to produce proper darkness of the specimen. (Slide Mounting Techniques for very small to microscopic animals) and recorded in a record of slide incubator drying (Nelson Riley C and Karin Gastreich, 2001).

5. Glass slides containing paraffin sections were stained by using conventional staining methods (Hematoxylin & Eosin staining) using an Automatic slide stainer following (Prepared Microscope Slides: Insects Specimen)

6. Take a glass slide with paraffin section at tissue staining and cover the slide. By covering it on the tissue, using a mounting medium to help fix the cover glass firmly on the slide. To promote easy and convenient microscopic examination and a good way to preserve specimens.

7. Once the biopsy slides are completed, they are analyzed under a microscope.

Results and Discussion

1. Studied morphology by microscope found the eggs of fly white colour, long and slim shaped size 0.2 mm.- 0.4 mm. Maggot is creamy white, the shape is slender and looks like a grain of rice. Body length about 0.6 cm.- 1.2 cm. Can be classified as Order Diptera (di = two, ptera = wings) refers to a group of insects with two wings. which are the flies (fly).

2. The general body shape has a pointed head and obtuse tail. The head has a mouth hook, and the tail has a pair of posterior spiracles (Jason H. Byrd and Jeffery K. Tomberlin, 2020).

3. Microscopic analysis of second and third stage maggots. Inside the posterior spiracle, two and three spiracular slits are found, respectively, Figure 4, Figure 5, and each piece is oval, fragment.

4. When examining by sectioning, to identify which family of maggots belong to the family, sectioning specimens must be processed for at least 1 day for each specimen. A complete slide will be provided to study the life cycle, habitat, and behavior of each maggot species identified for the purpose of estimating the time of death (Post Mortem Interval = PMI) as close to the truth as possible (minimum Post Mortem Interval = $_{min}$ PMI).

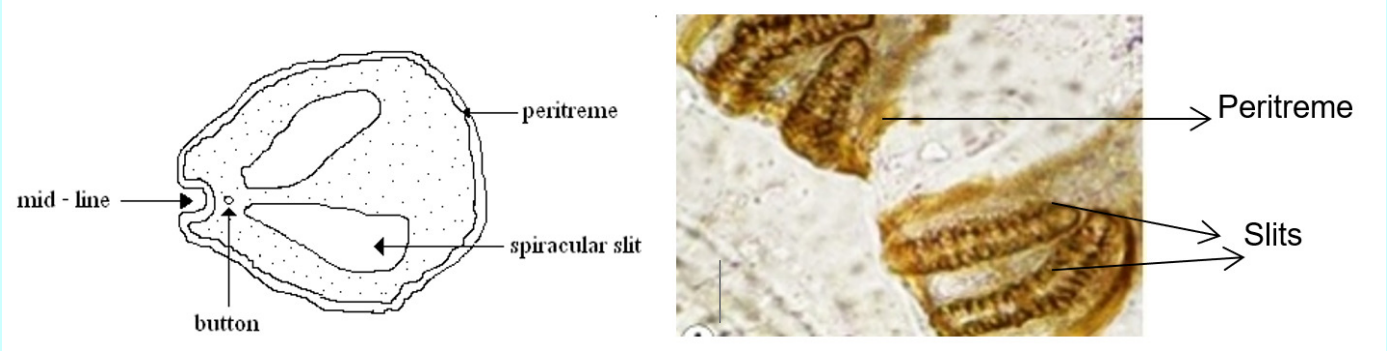


Figure 4: The posterior spiracle has two spiracular slits

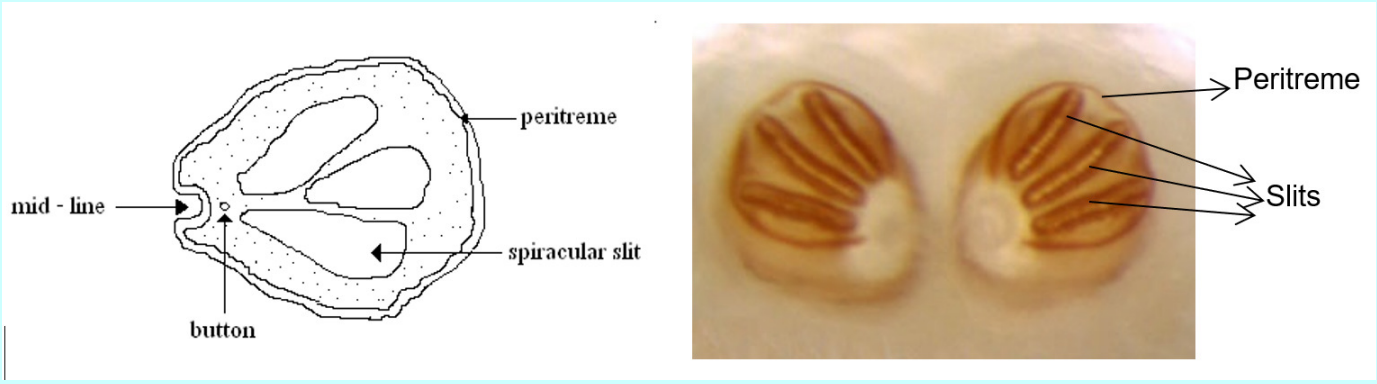


Figure 5: The posterior spiracle has three spiracular slits

Conclusion

Egg maggots and blow flies, second and third larvae of the Family Calliphoridae, Order Diptera, have a complete metamorphosis (Davies L. and Ratcliffe GG, 1994) with a total of 4 stages, namely egg stage, larva stage, pupa stage, and adult stage. The average blow fly life cycle is 6-8 weeks (Davies, 1994). The slower or faster life cycle of the blow fly depends on temperature, humidity, sunlight and the flies' food (Deonier, 1940). Blow flies prefer to lay their eggs on carcasses or on wounds, especially rotten wounds or on carcasses. Maggots are saprophyte, some live in dung, some are parasites of vertebrates. Some species feed on the blood of birds (avian) or others may inhabit or cause ulcers in humans or domestic animals (myiasis) (Zumpt, 1965). Blow flies are highly attracted to odors, such as the smell of human or animal corpses or the smell of blood, lymph, etc.

The presence of 2nd and 3rd maggots, blow flies on the corpse suggests that the corpse had been dead for at least 20 to 30 days, which is a forensic entomology estimate of mortality based on study the life cycle, behavior, and habitat of insects. Relatively to predict minimum time since death of Post Mortem Interval ($_{min}$ PMI), which is very useful in estimating mortality (Post Mortem Interval = PMI) after death (Davis JB and Goff ML, 2000).

References:

1. *Aballay, F. H., Domínguez, M. C., & Fernández Campón, F. (2012). Adult fanniidae associated to pig carcasses during the winter season in a semiarid environment: Initial examination of their potential as complementary PMI indicators. Forensic Science International, 219(1), 284.e1-284.e4. doi: 10.1016/j.forsciint.2011.11. 019.*
2. *Adams Z. J. O. and Hall M. J. R. 2003. Methods used for the killing and preservation of blowfly larvae and their effect on post-mortem length. Forensic Science International. 138(1-3), 50-61. doi: 10.1016/j.forsciint.2003.08.010.*
3. *3. The American Board of Forensic Entomology.(1996) The ABFE is the certifying Board for Forensic Entomologists in North America. Retrieved August, 30 2022, from https://forensicentomologist.org/.*
4. *Davies L. and Ratcliffe GG. (1994). Development rates of some pre-adult stages in blowflies with reference to low temperatures. Medical and Veterinary Entomology, 8(3), 245–254. doi: 10.1111/j.1365-2915.1994. tb00506.x.*
5. *Davis JB and Goff ML. (2000) Decomposition patterns in terrestrial and intertidal habitats on Oahu Island and Coconut Island, Hawaii. Journal of Forensic Sciences, 45(4), 836–842.*
6. *Deonier CC. (1940). Carcass temperatures and their relation to winter blowfly populations and activity in the Southwest. Journal of Economic Entomology, 33(1), 166–170. doi: 10.1093/jee/33.1.166.*
7. *Dorothy, E. Gennard. (2007). Forensic Entomology: An Introduction (pp.248). United Kingdom: John Wiley & Sons Ltd.*
8. *Forensic Science Services Act B.E. 2558 (2015), Bhumibol Adulyadej (2015, 3 August).*
9. *Jason H. Byrd and Jeffery K. Tomberlin. Forensic Entomology: The Utility of Arthropods in Legal Investigations 3rd Edition (pp.575). Australia: Taylor & Francis Group, LLC.*
10. *Lee, H.L. (1989). Recovery of Forensically Important Entomological Specimens from Human Cadavers in Malaysia – An Update. Malaysia Journal of Pathology, 11, 33-36.*
11. *Lee, G.M. and W.D. Lord. (1994). Entomotoxicology: A new area for forensic investigation. American Journal of Forensic Medicine and Pathology, 15(1), 51-57.*
12. *M. Lee Goff. (2000). A Fly for the Prosecution: How Insect Evidence Helps Solve Crimes (pp.240). United States: Harvard University Press.*
13. *Matuszewski S. (2021). Post-mortem interval estimation based on insect evidence Current challenges. Science & Justice. 12(4), 314. doi: 10.3390/insects1204 0314.*
14. *Nelson Riley C and Karin Gastreich . (2001). Permanently mounting Insects and other small Arthropods on microscope slides. Retrieved August, 30 2022, from https://biology.byu.edu.*

References (cont'd):

15. *Payne JA. (1965). A summer carrion study of the baby pig Sus scrofa Linnaeus. Ecology, 46(5), 592–602.*
16. *STEMpedia. (Year). Prepared Microscope Slides: Insects Specimen. Retrieved August, 30 2022, from https://thestempedia.com/tutorials/prepared-microscope-slides-insects-specimen/.*
17. *Rowe, Aaron. (2007). Bugs as Evidence. Law and Order, March, 55(3), 55-56.*
18. *Russell, W.C. (1947). Biology of the Dermestid Beetle with Reference to Skull Cleaning. Journal of Mammalogy, 28(3), 284-287.*
19. *Saigusa, Kiyoshi, Masataka Takamiya, Masatoshi Matsumasa, and Yasuhiro Aoki. (2006) The Forensic Availability of a Simple and Time Saving Method for the Identification of Dipteran Species to Estimate Postmortem Interval Using Entomological Evidence, (Letter to the Editor). Legal Medicine, 8(4), 203-254. doi: 10.1016/j.legalm ed.2006.04.004.*
20. *Sharma Ruchi, Rakesh Kumar Garg and J.R.Gaur. (2015) Various methods for the estimation of the post mortem interval from Calliphoridae: A review. Egyptian Journal of Forensic Sciences. 5(1), 1-12. doi:10.1016/j.ejfs.2013.04.002.*
21. *Sukontason, Kabkaew L. , K. Sukontason, P. Narongchai, S. Lertthamnongtham, S. Piangjai and J. K. Olson. 2001. Chrysomya rufifacies (Macquart) as a forensically-important fly species in Thailand : A case report. Journal of Vector Ecology. 26(2) : 162-164.*
22. *Thomas, D.B., and A.C. Chen. (1989). Age Determination in Adult Screw-Worms (Diptera: Calliphoridae) by Pteridine Levels. Journal of Economic Entomology. 82(4), 1140-1144. doi: 10.1093/jee/82.4. 1140.*
23. *Universiti Teknologi MARA (UiTM). (2014). "Blowfly maggots provide physical evidence for forensic cases." ScienceDaily. Retrieved August, 30 2022, from www.sciencedaily.com/rel eases/2014/09/140903133125.htm.*
24. *Zumpt, F. (1965). Myiasis in Man and Animals in the Old World. United Kingdom: Butterworths.*

Case Study

Identification of Liquid Methamphetamine Smuggled in Gasline Jerrycans: A Diversion Method of Drugs Trafficking

Mrs. Tanti, Mrs. Carolina Tonggo Marisi Tambunan, Mrs. Maimunah, Mrs. Christien Andriyani Lalangi, Mrs. Dwi Handayani, Mrs. Erlana Nindya Maulida, Mrs. Verawati Rahman, Mrs. Widianti Ningtias, Mr. Martin Luther Silubun, Mrs. Utari Pramudita, Mr. Andre Hendrawan*
Center of Drugs Testing Laboratory of National Narcotics Board (BNN), Republic of Indonesia
**Email: tanti.puslabbnn@gmail.com, drugslaboratory@bnn.go.id*

Introduction

Illicit drug traffickers transport their products entering Indonesia using a wide array of innovative smuggling methods primarily via maritime routes. Methamphetamine can be smuggled in the form of either packaged methamphetamine or methamphetamine in solution [1]. In our country, methamphetamine is primarily seized in the packaged crystal form with a total amount of 9.2 tons of methamphetamine seizures in 2023 [2]. DEA reported many cases of liquid methamphetamine transported from one place to another using tankers, large capacity barrels, or car and truck fuel tanks across the United States. It is easier to smuggle and more difficult to detect since it can be mixed with other liquids, making it possible to smuggle in larger quantities. However, conversion of liquid methamphetamine to the crystal form is required prior to distribution [3]. As a new record, the Jambi Regional Police of the Republic of Indonesia succeeded in uncovering a method of diversion for smuggling liquid methamphetamine in one of five fuel-filled jerrycans. In this article, sample handling and the findings of laboratory analysis will be presented.

Case

In May 2023, five blue jerrycans with a capacity of 75 L contained liquids for potential presence of methamphetamine with a total gross weight of 264.730 kg were seized by Jambi Regional Police in Banten waters, Indonesia (Figure 1).

The evidence was shipped by speed boat involving one Iranian as a suspect.

An average of 27.8 milliliters of liquid from each jerrycan in code: 1A, 2A, 3A, 4A, and 5A were submitted to BNN Laboratory for analysis.

Figure 1: Five blue jerrycans contained liquids seized by Jambi Regional Police

Materials and Methods

1. Reagents and standards

All reagents and standards used in the analysis were obtained from Merck (Germany) and Lipomed (Switzerland), respectively.

2. Sample treatment

All samples were physically observed as a preliminary analysis. There was a slight color difference of five investigated liquids (Figure 2), where sample 4A appeared more colorless than others. The odor of samples was likely fuels, except sample 4A. Liquid in code 4A showed another uniqueness since it had a higher viscosity than others. The average mass-to-volume ratio for the four samples (1A, 2A, 3A, and 5A) was 0.7103 kg/l, while for sample 4A was 1.0578 kg/l. It might be related to the viscosity properties of sample 4A, since the viscosity and density relationship was reported to be linear [4].

Color tests using Marquis, Mandelin, and Simon were directly performed on the liquids. Sample 4A showed a different color change from the others where the dark blue color in Simon's reagent indicated the presence of secondary amine in the sample [5]. It was suspected to contain methamphetamine, although the color changes of the Marquis and Mandelin reagents did not fit the characteristic, possibly due to liquid interference.

Figure 2: (a) Color variations of five liquid samples

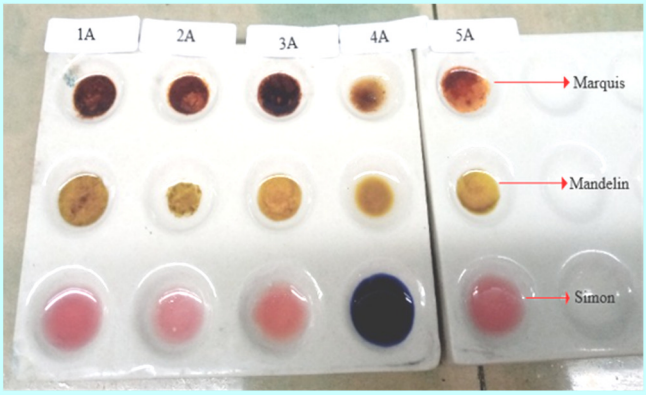


Figure 2: (b) Color test of five liquid samples

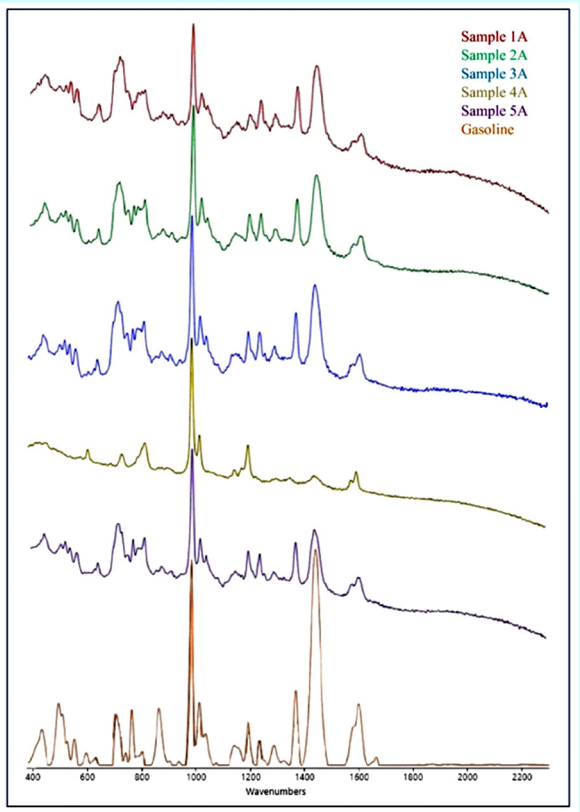


Figure 3: Raman spectra of five investigated samples and Gasoline (database)

Chemical analysis technique was initially performed by The Metrohm Instant Raman Analyzer (MIRA) DS with wavelength of 785 nm and spectral range of 400 to 2300 cm^{-1}. The results are shown in Figure 3 and Table 1.

Figure 3 differentiates between sample 4A and the others when compared to the spectra of gasoline. Methamphetamine (92%) was detected as the main compound of sample 4A with no gasoline as the basis of liquid. Overlayed of Raman spectra sample 4A and methamphetamine was very identical (Figure 4), shown by the Hit Quality Index (HQI) score close to 1.

Table 1: HQI match scores and spectral weight % of five investigated samples

Sample Code	Compound Detected	Match Score (HQI*)	Spectral Weight %
1A	Gasoline	NA**	57%
2A	Gasoline	0.80	73%
3A	Gasoline	0.79	59%
4A	Methamphetamine	0.97	92%
5A	Gasoline	NA**	72%

*) HQI: Hit Quality Index
**) NA: Not Available

On the other hand, liquids in samples 1A, 2A, 3A and 5A were detected as gasoline and did not contain any illicit drug. Reported HQI values of gasoline from four samples were either under the assigned threshold of 0.85 [6] or even not available. Further analysis might be required to ensure the presence of gasoline in those liquids, but the mass and volume ratio of the measured samples was similar to the reported density of gasoline, which is around 0.70 - 0.75 kg/l [7]. It strengthened the suspicion that the liquids in samples 1A, 2A, 3A and 5A were gasoline.

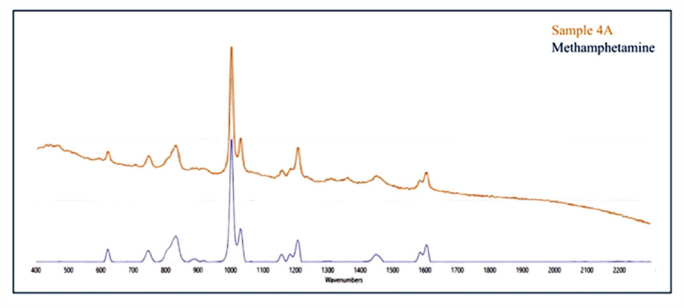


Figure 4: Overlayed of Raman spectra Sample 4A and Methamphetamine (database)

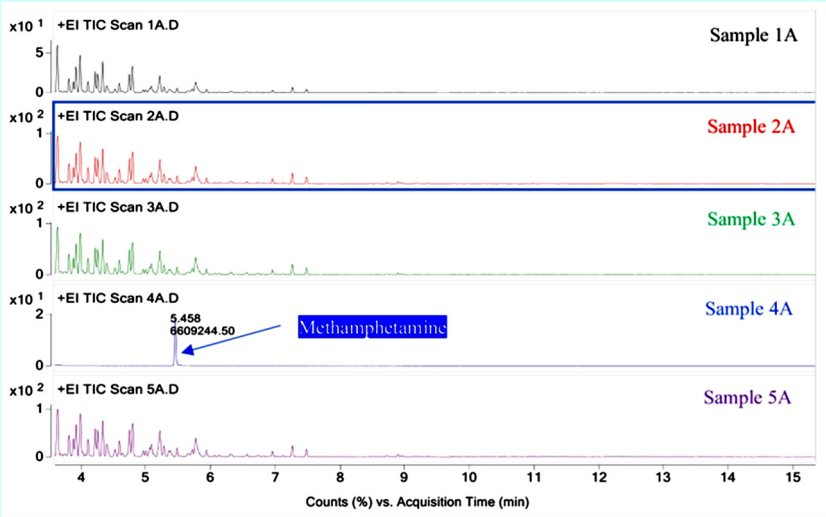


Figure 5: GC-MS analysis results of the five investigated liquids

A confirmation test was carried out using GC-MS Agilent 7890B-5977B to identify the contents of five investigated liquids. A HP-5MS column (30 m x 0.25 mm x 0.25 µm) was used with He carrier gas. GC-MS operating conditions were adapted from [8]. Two milliliters liquid of each code was mixed homogeneously with 8 mL of aquabidest prior to liquid-liquid extraction. The GC-MS analysis confirmed that only sample 4A contained methamphetamine, which was consistent with the result of Raman analysis (Figure 5).

To extract methamphetamine from sample 4A, 20 mL of liquid was evaporated to dryness under constant nitrogen flow. White crystal was formed right after the liquid vaporized. Crystal was dried in the oven at 70°C to remove the remaining liquid. The same treatment was carried out on other samples, no crystals were formed until the evaporation stopped.

3. Crystal analysis

3.1 Screening test

Formed-crystal (Figure 6b) was tested using Marquis, Mandelin, and Simon reagents to determine the presence of methamphetamine.

3.2. Confirmation test

Analysis was carried out using GC-MS. Sample was prepared by dissolving 1 mg of crystal in 3 mL methanol.

3.3. Purity test

Lipomed reference stamethamphetamine.HCl were dissolved in methanol in 5 concentration levels, each in triplicates, to build a calibration curve. Methamphetamine purity ndard of *d,l*-analysis was performed by Shimadzu GC-2010 with FID and AOC-5000 Autoinjector. One µl of 100 µg/mL samples (triplicates) in methanol were injected to GC-FID using the DB-5 column, 30 m x 0.25 mm ID x 0.25 µm. Separation was achieved using the following oven temperature program: 135°C (2 min) as initial temperature with 15°C/min to 230°C. Flow rate was set on 1.2 mL/min. The injector and detector temperature were set at 280°C and 300°C, respectively.

(a) (b)

Figure 6: White crystal formed in sample 4A: (a) after evaporation; (b) after oven-drying

Results and Discussion

Methamphetamine in sample 4A was extracted from the dissolving liquid and recrystallized prior to further analysis. Recrystallization technique used was quite simple, by allowing the crystal to dry completely. Nitrogen evaporation of 20 mL liquid from sample 4A followed by oven drying obtained a total of 12.9346 grams of white crystal, equivalent to 64.67% w/v. Total volume of liquid in the blue jerrycan code 4A was 54.11 L. It means that approximately 35 kilograms of crystal methamphetamine could be obtained when all the liquid in the jerrycan 4A is converted into solid form.

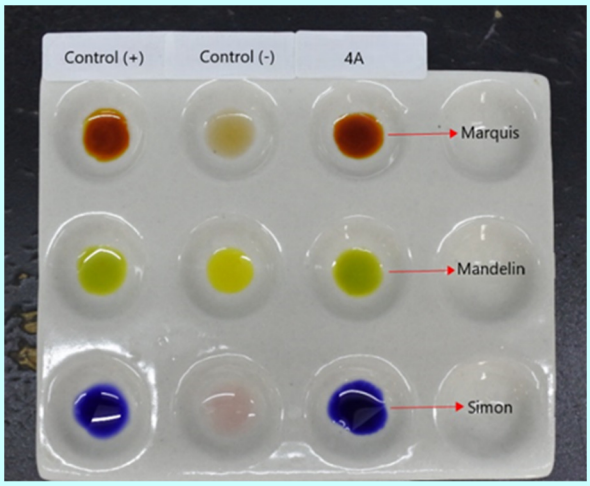


Figure 7: Color test of recrystallized crystal by liquid evaporation and oven-drying

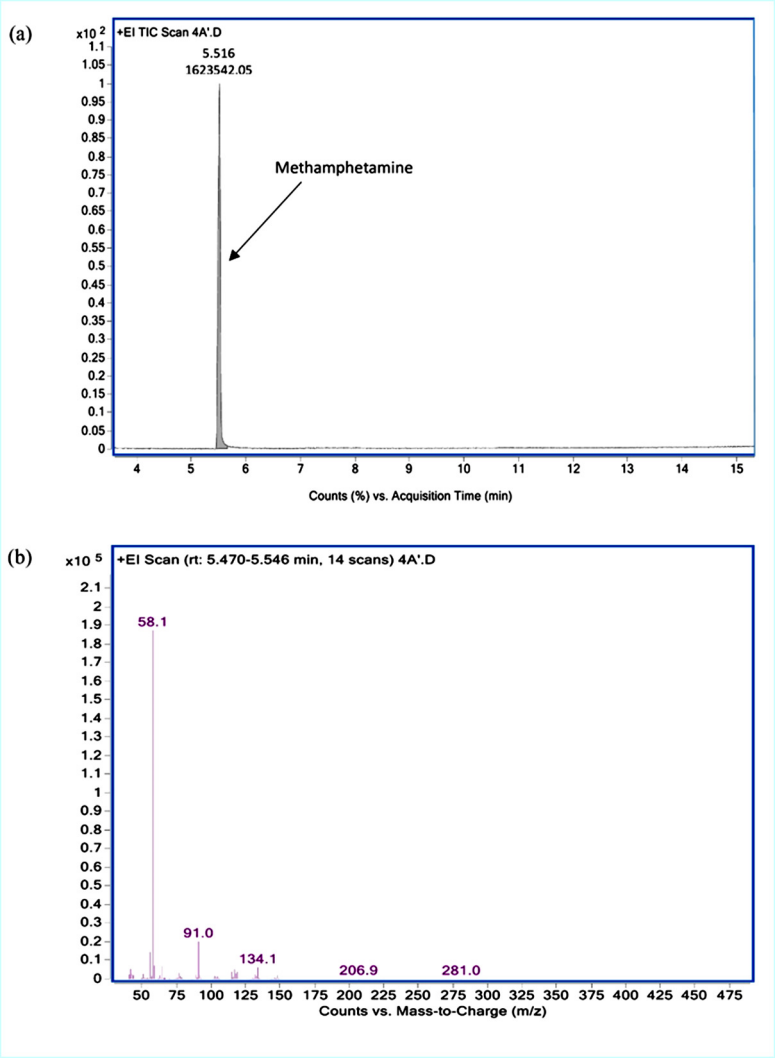


Figure 8: GC-MS analysis result of the recrystallized crystal:
(a) GC chromatogram (full scan mode);
(b) *EI-MS spectrum of* methamphetamine

A color test using Marquis, Mandelin, and Simon's reagents was applied to the crystal and showed the specific color changes for methamphetamine as described by UNODC [5] (Figure 7). Liquid evaporation removed the liquid-base interference and increased the sample purity, resulting in clearer color changes to each reagent compared to the color test result of liquid in sample 4A (Figure 2b).

A confirmation analysis of crystal using GC-MS showed a sharp single peak in retention time of 5.516 min with major fragment ions of m/z 58, 91, 56, 134 (Figure 8a,b). The peak was confirmed as Methamphetamine by its mass fragmentation and comparison to mass spectral libraries.

Purity analysis was carried out by GC-FID. The GC-FID was calibrated prior to sample analysis. One milligram of crystal methamphetamine diluted in 10 mL methanol. The solution was filtered using 0.2 µm PTFE syringe filter before injection. The following equation was used to calculate the purity of methamphetamine recrystallized from liquid in sample 4A.

$$\frac{a \times V}{w} \times 100\%$$

In which **a** is the calculated concentration of methamphetamine in mg/mL, **V** is the final volume of sample preparation in mL, and **w** is the weight of crystal in mg.

The measured purity of methamphetamine.HCl was 96.02%.

Conclusion

A diversion method of smuggling liquid methamphetamine in one of the five blue jerrycans containing gasoline belongs to a unique case that we handled. A total of 12.9346 grams of white crystal methamphetamine was recrystallized after 20 mL of liquid were evaporated with nitrogen and then oven-dried. When the whole volume of liquid in the jerrycan was turned into solid form, it would be comparable to about 35kg of crystal methamphetamine. Recrystallization technique that applied to the sample resulting in a purified crystal of methamphetamine.HCl with purity of 96.02%.

Acknowledgement

1. Police Brigadier General Supiyanto, Head of Center of Drugs Testing Laboratory of National Narcotics Board of the Republic of Indonesia
2. Jambi Regional Police of the Republic of Indonesia
3. Drugs Profiling Team of Center of Drugs Testing Laboratory of National Narcotics Board of the Republic of Indonesia

References:

1. *Drug Enforcement Administration. 2020 National Drug Threat Assessment (NDTA). United States: Drug Enforcement Administration's (DEA) Intelligence Program; 2020. https://www.dea.gov/documents/2021/03/02/2020-national-drug-threat-assessment. (accessed March 07, 2025).*
2. *National Narcotics Board. Indonesia Drug Report 2024. Jakarta: Research, Data and Information Center National Narcotics Board; 2024. https://puslitdatin.bnn.go.id/konten/unggahan/2024/07/IDR-2024-Versi-Inggris_FINAL_opt.pdf. (accessed March 07, 2025).*
3. *United States Sentencing Commission. Methamphetamine Trafficking Offenses in the Federal Criminal Justice System. Washington DC: United States Sentencing Commission; 2024. https://www.ussc.gov/research/research-reports/methamphetamine-trafficking-offenses-federal-criminal-justice-system. (accessed March 07, 2025).*
4. *Hussein MJ, Mohammed KA, Naser FH. Assessment of excavation slurry produced clayey mineral from the Bahr Al-Najaf region. Pollack Periodica. 2024 Mar 22;19 (1):111-7.*
5. *United Nations Office Drugs and Crime. Colour Test for Precursor Chemicals of Amphetamine-Type Substance The Use Colour Test for Distinguishing between Ephedrine-Derivatives. Hungary: Scientific and Technical Notes; 2005. https://www.unodc.org/pdf/scientific/SCITEC20-fin.pdf. (accessed March 11, 2025).*
6. *Gelwicks M. Verification, p-values, and Training Sets for the Mira P. 2018. https://www.metrohm.com/content/dam/metrohm/shared/documents/white-papers/WP-024EN.pdf. (accessed March 10, 2025).*
7. *Speight JG. Production, properties and environmental impact of hydrocarbon fuel conversion. In Advances in clean hydrocarbon fuel processing. Woodhead Publishing. 2011, Jan:54-82. https://doi.org/10.1533/9780857093783.1.54. (accessed March 11, 2025).*
8. *United Nations Office on Drugs and Crime. Recommended methods for the identification and analysis of amphetamine, methamphetamine and their ring-substituted analogues in seized materials. New York: United Nations Publication; 2006. https://www.unodc.org/pdf/scientific/stnar34.pdf. (accessed March 11, 2025).*

Case Study

Beyond The Cloud: Forensic Insights into Singapore's Vape Landscape

Tan Ying Ying, Dr Larissa See Kum Foong**, Dr Ong Mei Ching, Lim Jong Lee Wendy*
Health Sciences Authority, Singapore
*Email: *Tan_Ying_Ying@hsa.gov.sg, **Larissa_see@hsa.gov.sg*

Vape Scene in Singapore

The Vaping has emerged as a global phenomenon, and Singapore is no exception to this trend. Despite stringent laws in Singapore prohibiting the import, sale and use of vape devices and related products, the prevalence of vaping continues to rise [1], particularly among the younger population[2,3]. Youths are drawn to vaping due its perceived trendiness, the misconception that it is less harmful compared to smoking traditional cigarettes and the appeal of fanciful vape designs and their enticing flavours. The proliferation of social media and online platforms have further facilitated the illegal distribution of vape products, making them increasingly accessible to users despite the legal restrictions. Compounding the issue is the increasing use of vape devices as delivery systems for other drugs of abuse and toxic chemicals, posing significant challenges to both public health and law enforcement. The present article reviews the vaping products encountered in Singapore along with an overview of their analysis and selected case studies.

Types of Vaping Devices

The vape devices analysed by our laboratory have grown increasingly complex and sophisticated in design and functionality over the years. The common types [4] encountered (refer to Figure 1) include:

a. Vape Pens - Single-use devices pre-filled with e-liquids, designed to be discarded after liquid or battery has depleted
b. Vape Pods – Compact vaping devices featuring refillable or pre-filled e-liquid cartridge that can be replaced once empty

Controlled Drugs and Toxic Chemicals in Vape Products

The content in vape products has evolved significantly over the past decade [5-8]. While nicotine-based e-liquids remain the most common type in Singapore, there is an emerging trend of e-liquids being adulterated with controlled drugs, new psychoactive substances (NPS) and toxic chemicals such as vitamin E acetate. In 2024, local authorities uncovered several "Kpods" - vape juice mixed with etomidate. However, incidents involving controlled drugs concealed within vape products still account for a minority of all drug seizures.

Cannabis-based e-Liquids

Cannabis-based e-liquids seized in Singapore are infused with cannabinoids such as tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD) and semi-synthetic cannabinoids (SSCs) including THC acetate and hexahydrocannabinol (HHC), first detected in vape products in 2023 and 2024, respectively. Unlike nicotine-based e-liquids, cannabis-based variants are more viscous, lack humectants and may contain vitamin E acetate as an oil-based cutting agent. This compound has been identified as a potential toxin of concern due to its ability to accumulate in the lungs, causing severe respiratory issues and long-term lung injury [9].

Figure 1: Vape pens (left) and vape pods (right)

Figure 2: Viscous liquid from a vape pen containing delta-9-THC, delta-8-THC and vitamin E acetate

Figure 3: Vape pen containing delta-9-THC, delta-8-THC, delta-8-THC acetate and CBN

Figure 4: Vape pen containing delta-9-THC, CBD and CBN

NPS-containing e-Liquids

E-liquids adulterated with new psychoactive substances (NPS) were first detected in Singapore in 2020. Since then, synthetic cannabinoids such as MDMB-4en-PINACA, MDMB-BUTINACA, MDMB-INACA, ADB-INACA and ADB-4en-PINACA have been identified in such products. MDMB-INACA and ADB-INACA may be precursors used for the synthesis of MDMB-4en-PINACA and ADB-4en-PINACA, respectively.

Figure 5: Vape pods containing MDMB-4en-PINACA and MDMB-BUTINACA

Figure 6: Vape pens containing ADB-INACA and ADB-4en-PINACA

E-liquids containing other Controlled Drugs and Etomidate

In addition to cannabis-based and NPS-containing e-liquids, other controlled drugs in Singapore have also been identified in vape products. These include low concentrations of methamphetamine and ketamine, often found in combination with nicotine.

In 2024, e-liquids containing etomidate [10], an ultra-short-acting intravenous anaesthetic, typically used in medical procedures had emerged. Most of these samples were found to contain etomidate as the sole active ingredient, while a small proportion were found to contain additional substances such as metomidate, etomidate propyl analogue, nicotine, and other controlled drugs (e.g. synthetic cannabinoids, methamphetamine and ketamine).

Although etomidate and its analogues are currently not controlled in Singapore, other countries have taken regulatory action [11]. In China and Hong Kong SAR, etomidate and some of its analogues have been placed under control due to concerns over its increasing prevalence and misuse.

Figure 7: Vape pod containing nicotine and methamphetamine

Figure 8: Vape pod containing etomidate and ketamine

Figure 9: Vape pod containing etomidate

Analytical Workflow for Vape Analysis

Sample Preparation

The analytical workflow for vape analysis in our laboratory is shown in Figure 10.

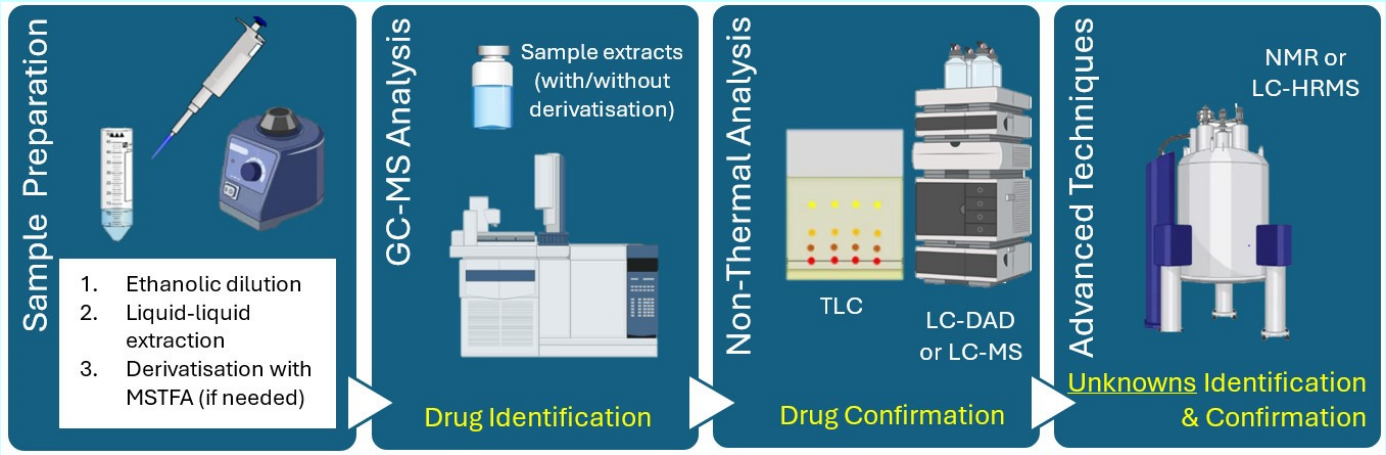


Figure 10: Workflow for vape analysis

The analytical workflow begins with sample preparation involving either:

a. Dilution with Absolute Ethanol

A simple dilution step with absolute ethanol is applied to e-liquids which are viscous and sticky, such as the cannabis-based e-liquids. A small amount of sample (~50 milligram) will be diluted with 5 mL of absolute ethanol.

The analytical workflow begins with sample preparation involving either:

b. Liquid-Liquid Extraction

Basic extraction using purified water, sodium hydroxide solution and ethyl acetate is used for e-liquids containing glycerol, such as nicotine-based e-liquids. 1 mL of sample will be diluted with 4 mL of purified water. An appropriate volume of 1N sodium hydroxide is then added to adjust the pH of the sample solution to 10-12, before extracting with 2 mL of ethyl acetate. This extraction procedure serves as a sample clean-up step by removing or reducing the amount of glycerol, which could otherwise mask the detection of targeted drugs, as illustrated in Figure 11 below.

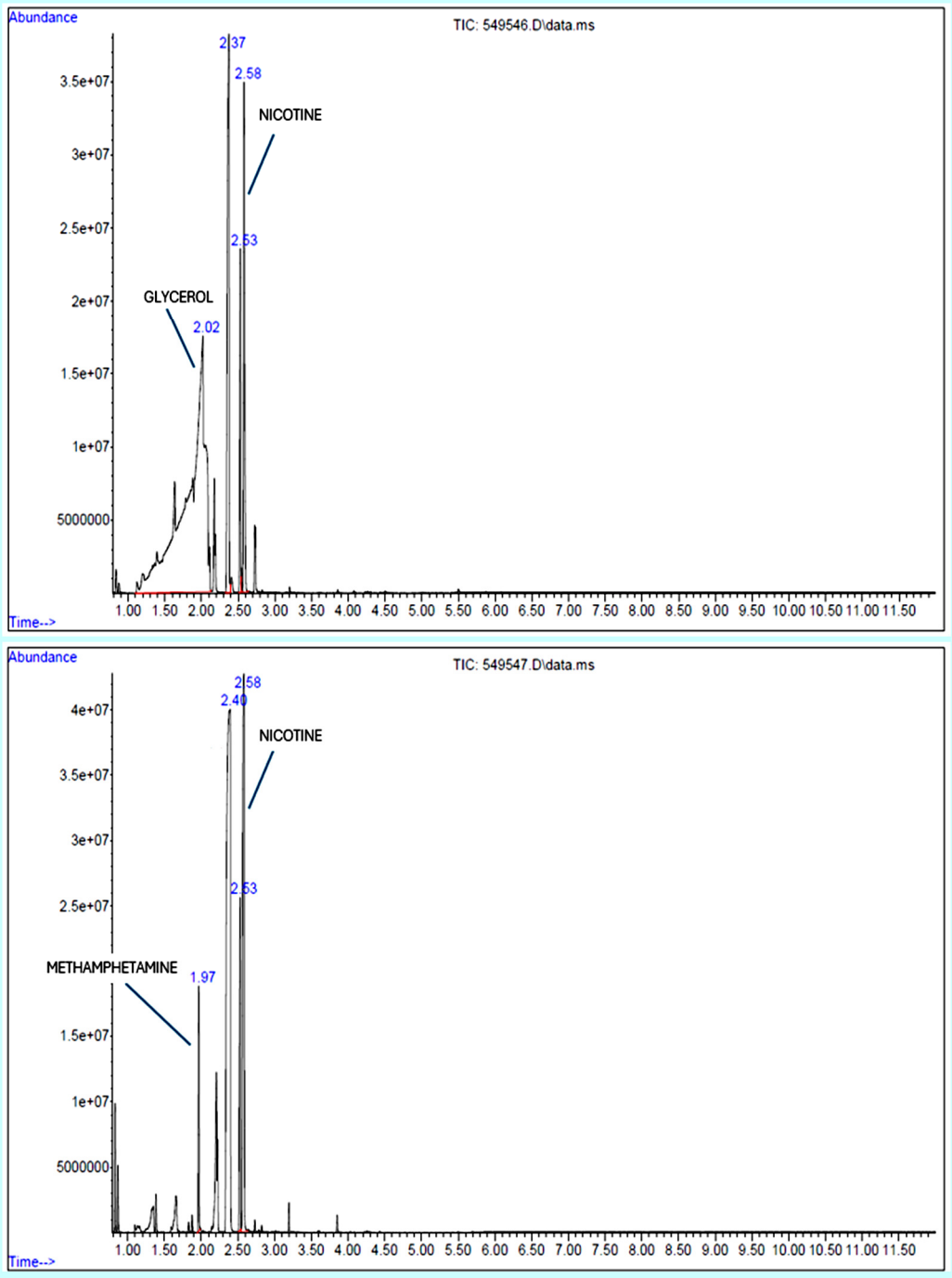


Figure 11: GC-MS chromatogram of an e-liquid containing nicotine and methamphetamine extracted using absolute ethanol (top) and basic extraction (bottom)

For e-liquids containing closely related isomers, such as delta-6a,10a-THC, delta-8-THC and delta-9-THC, derivatisation with N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) may also be performed to improve chromatographic separation during instrumental analysis. 150 µL of the ethanolic extract is evaporated to dryness and reconstituted in 200 µL petroleum ether. 50 µL of the derivatising agent, MSTFA, is then added to the solution. The resulting mixture is vortexed and left to stand for 30 minutes before GC-MS analysis. An example is shown in Figure 12 below where enhanced peak separation could be observed after derivatisation with MSTFA.

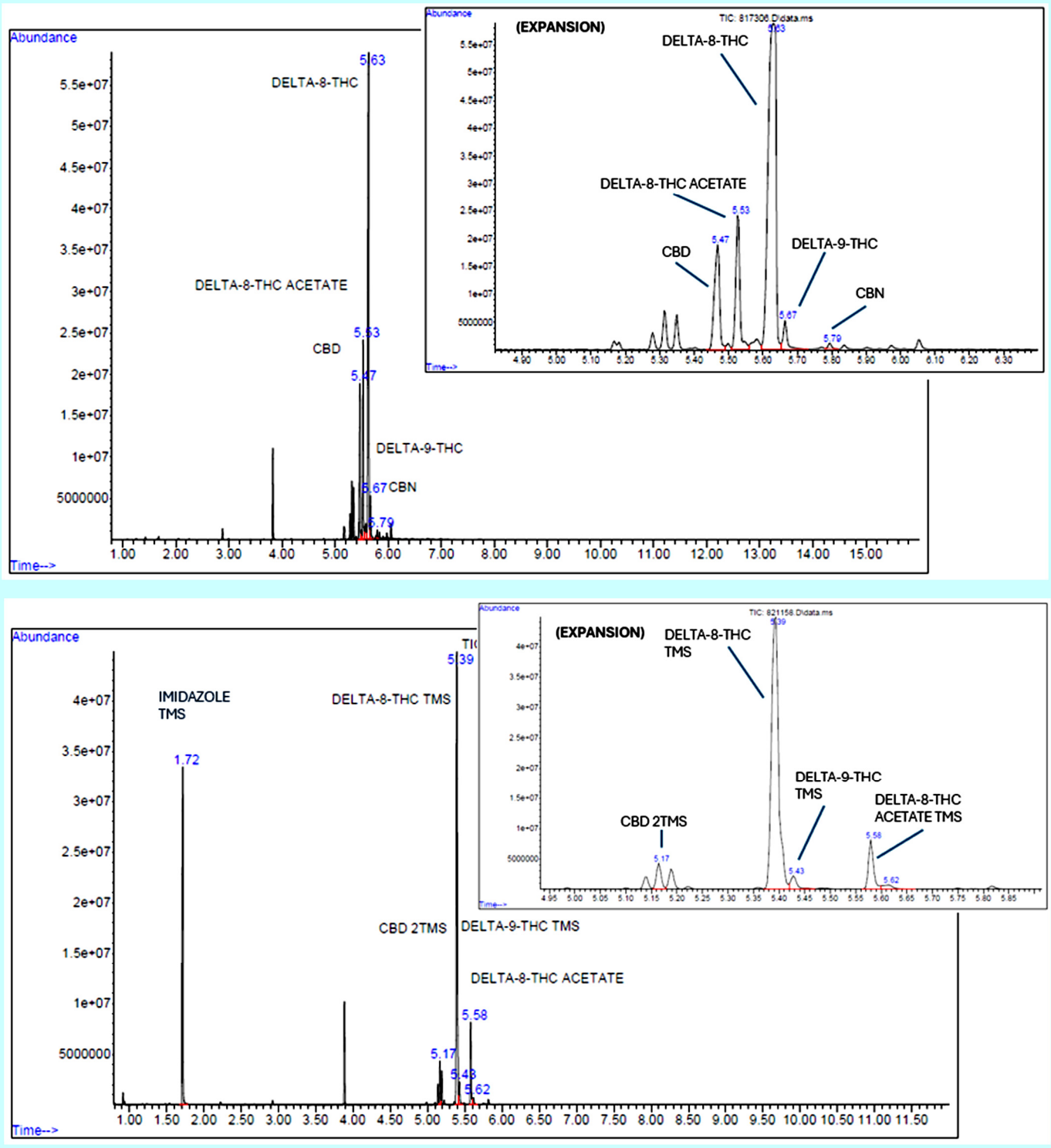


Figure 12: GC-MS chromatogram of an e-liquid containing delta-8-THC, delta-8-THC acetate and delta-9-THC before derivatisation (top) and after derivatisation (bottom) with MSTFA

Additionally, this derivatisation procedure is effective for the identification of acidic cannabinoids such as tetrahydrocannabinolic acid (i.e. THCA), which would otherwise decarboxylate to their neutral forms under high temperatures used in GC-MS analysis. Figure 13 illustrates how THCA can be detected alongside THC after MSTFA derivatisation.

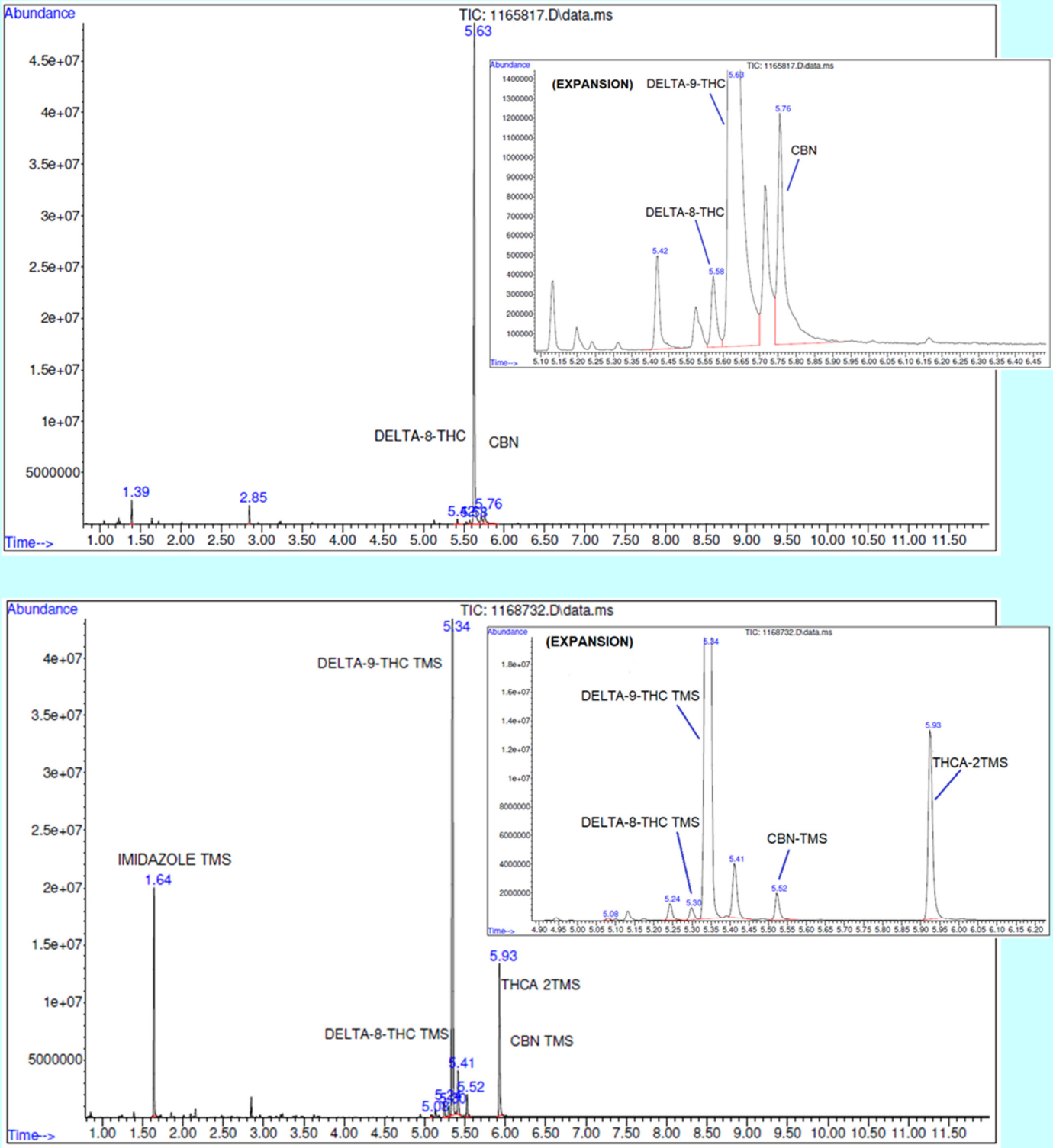


Figure 13: GC-MS chromatogram of an e-liquid containing delta-8-THC, delta-9-THC, delta-9-THCA and CBN before derivatisation (top) and after derivatisation (bottom) with MSTFA

Instrumental Analysis

Gas chromatography-mass spectrometry (CG-MS) is the primary technique used by our laboratory to separate and identify compounds of interest. For samples having complex matrices, method optimisation including the use of different column chemistries (e.g. HP-5 or DB-35), column lengths and temperature programs may be necessary to achieve better peak resolution.

For thermally labile compounds such as THCA which can degrade to THC and CBN under high temperatures, complementary analysis using non-thermal techniques such as liquid chromatography-diode array detection (LC-DAD), liquid chromatography-mass spectrometry (LC-MS) or thin layer chromatography (TLC) are also performed to confirm their drug identities.

For novel substances lacking drug reference standards, analysis using advanced instrumental techniques such as liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS) and nuclear magnetic resonance (NMR) can be used for structural elucidation.

Case Studies

The chemical composition of vape products submitted to our laboratory has been highly variable. Below are some interesting GC-MS profiles observed from analysed samples.

Table 1: GC-MS method parameters used for vape analysis

GC Conditions	
Column	Agilent HP-5MS, 12.5 m × 0.2 mm *i.d.* × 0.33 µm
Oven Temp	80°C (0.5 min) à 300°C (6 mins) at 40°C/min
Solvent Delay	0.7 min
Inlet Temp	280°C
Carrier Gas	Helium
Column Flow	1.6 mL/min (constant flow)
Interface Temp	300°C
Run Time	12 mins
Injection Parameters	Split mode: Injection volume = 0.5 µL to 1 mL Split Ratio: 40:1 or 70:1

MS Parameters (EI tuned to PFTBA)	
Electron energy	70 eV
Interface Temp	280°C
Scan Rate	2^N where N = 1
Scan Range	35-600 amu
Ion Source Temp	230°C
Quadrupole Temp	150°C
Gain Factor	1

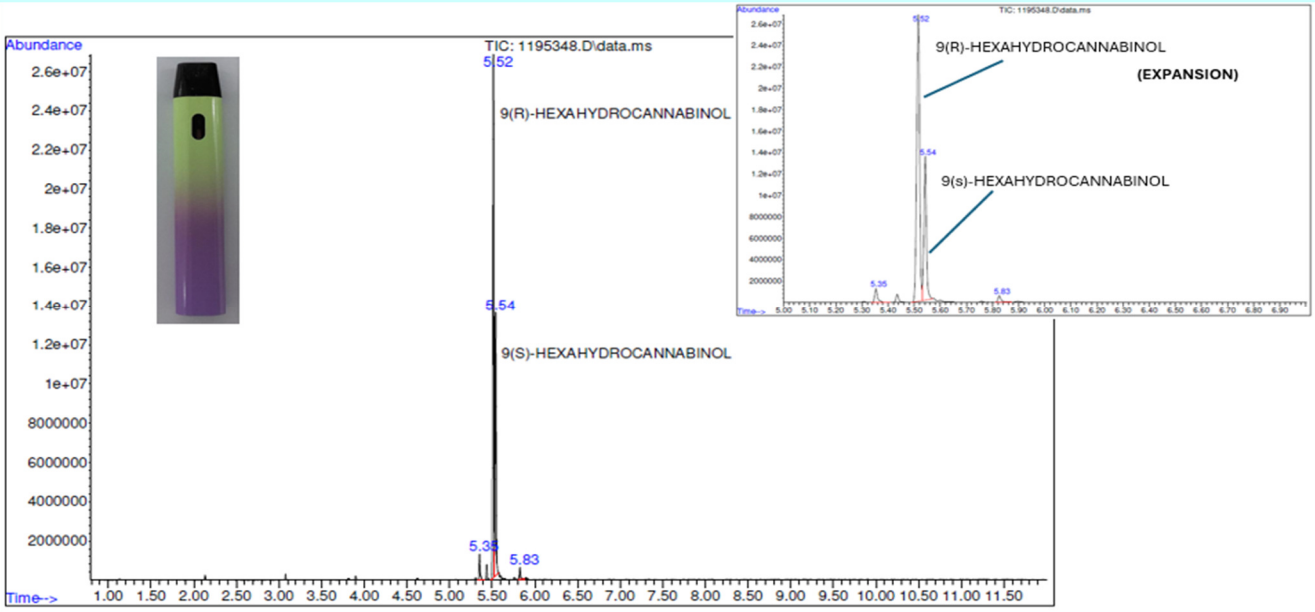


Figure 14: GC-MS Chromatogram of an e-liquid containing hexahydrocannabinol (HHC)

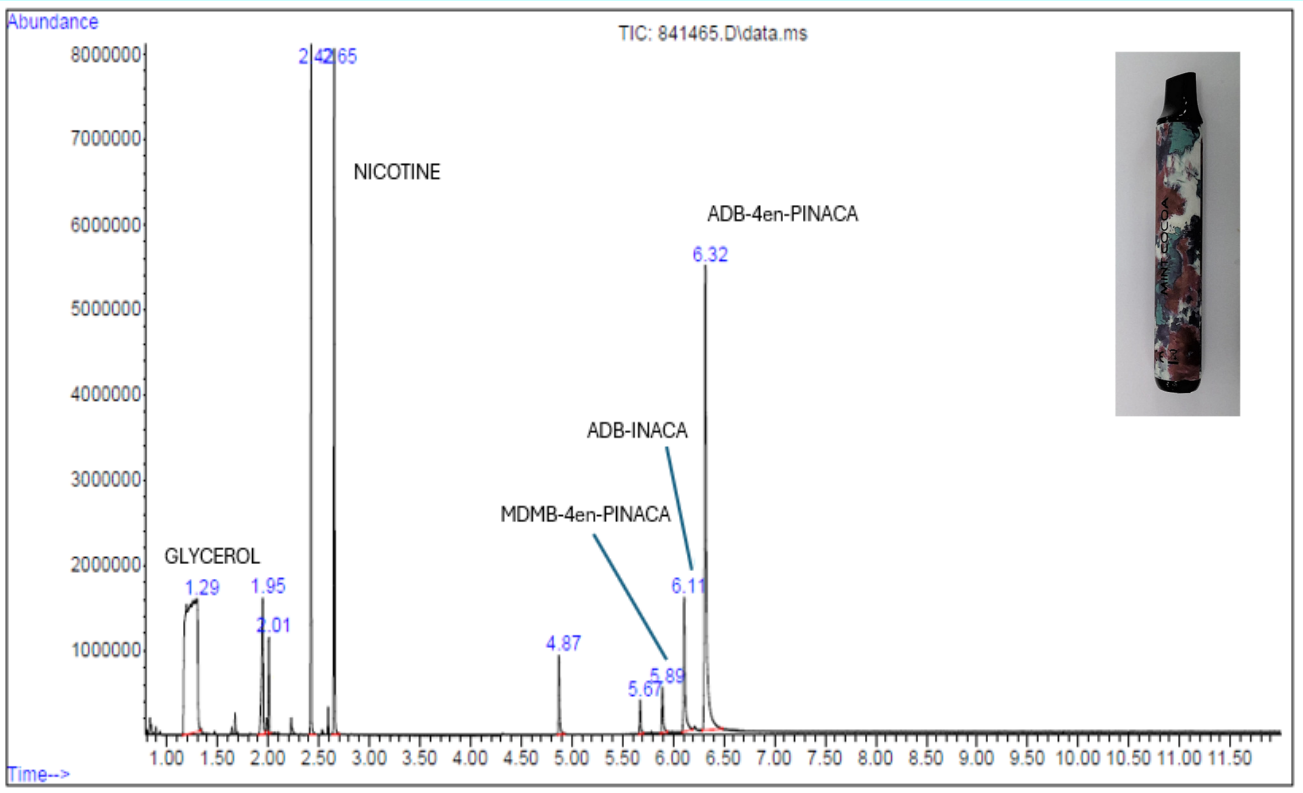


Figure 15: GC-MS Chromatogram of an e-liquid containing synthetic cannabinoids, MDMB-4en-PINACA, ADB-INACA and ADB-4en-PINACA

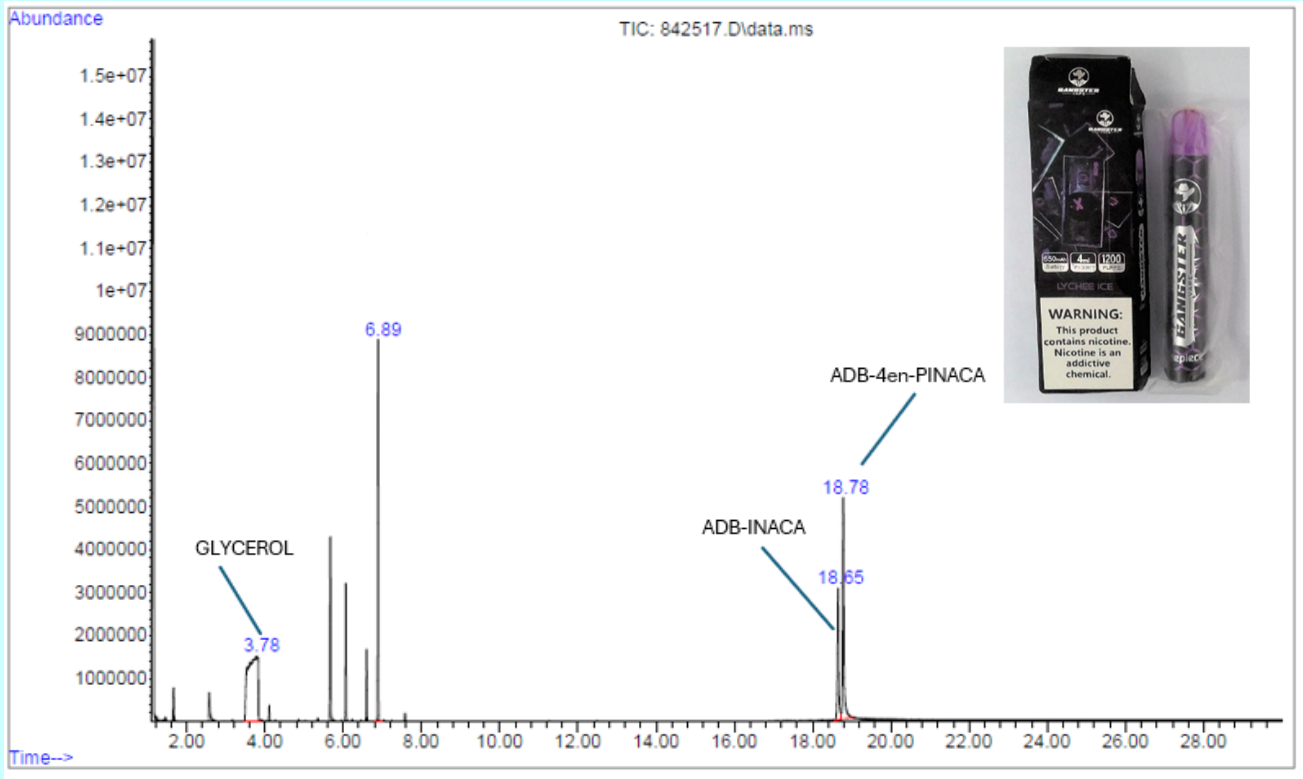


Figure 16: GC-MS Chromatogram of an e-liquid containing synthetic cannabinoids, ADB-INACA and DB-4en-PINACA

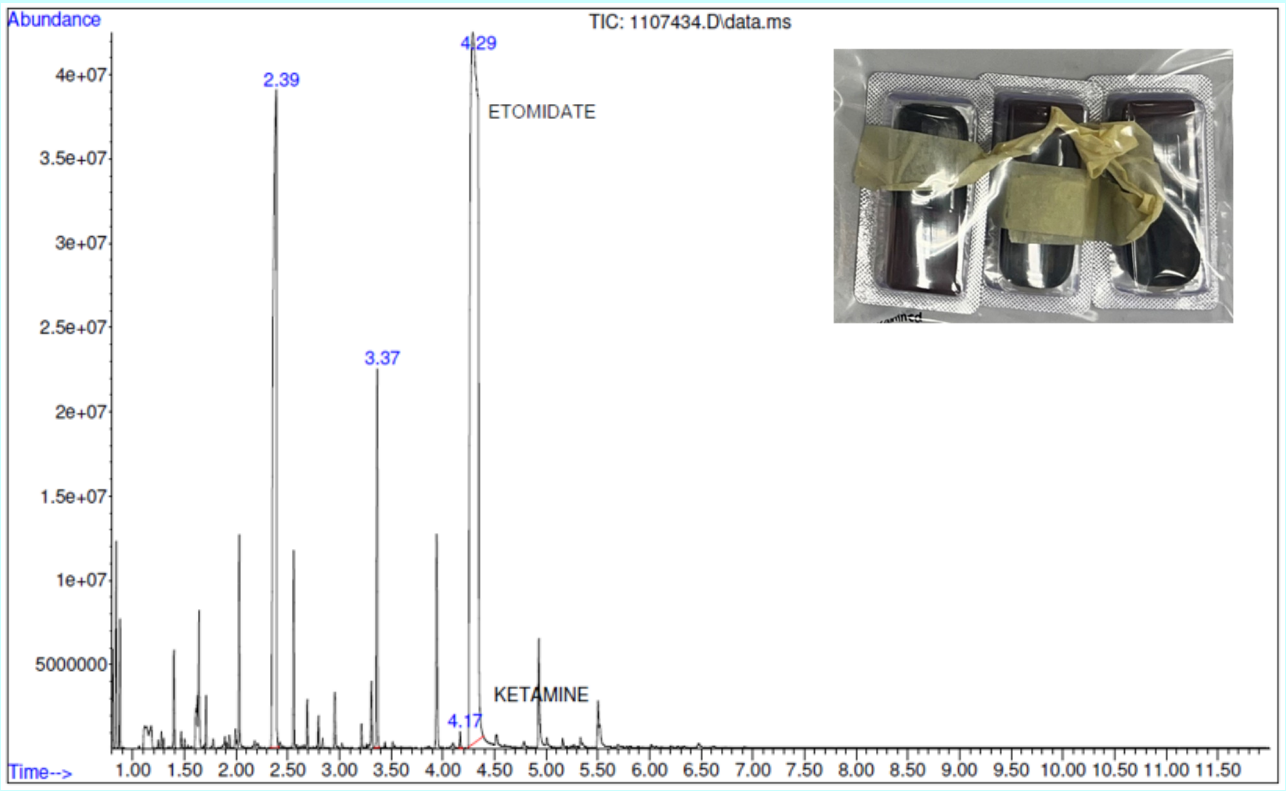


Figure 17: GC-MS Chromatogram of an e-liquid containing etomidate with trace amount of ketamine

Conclusion

Vaping continues to be a growing concern in Singapore despite strict laws due to its perceived trendiness and the growing availability and diversity of illicit vape products. The variability and complexity of these products due to adulteration with controlled drugs, new psychoactive substances and toxic chemicals such as vitamin E acetate present serious public health concerns and challenges for law enforcement. To keep pace with the fast evolving vape landscape, laboratories must develop robust testing methodologies capable of detecting and accurately identifying drugs of interest within the complex matrices of vape products.

References:

1. *The rise of vaping in Singapore, explained, Chanel News Asia, 4 Aug 2024 (https://www.channelnewsasia.com/singapore/vape-vaping-cna-explains-hsa-moe-moh-4519186).*
2. *Vaping among youth in Singapore: The real damage it is doing to their bodies, The Straits Times, 15 Apr 2024 (https://www.straitstimes.com/singapore/vaping-among-youth-in-singapore-the-real-damage-it-is-doing-to-their-bodies).*
3. *Police alerted HSA to over 4,000 cases of vaping from 2023 to 2024, The Straits Times, 4 Mar 2025 (https://www.straitstimes.com/singapore/politics/police-alerted-to-over-4000-cases-of-vaping-from-2023-to-2024-1-parent-reported-child).*
4. *About E-cigarettes (Vapes), CDC, 24 Oct 2024 (https://www.cdc.gov/tobacco/e-cigarettes/about.html).*
5. *C. Giroud, M. Cesare, A. Berthet, V. Varlet, N.C. Lozano, B. Favrat, E-Cigarettes: A review of New Trends in Cannabis Use Int. J. Environ. Res. Public Health, 2015.*
6. *M.S. Blundell, P.I. Dargan, D.M. Wood, The dark cloud of recreational drugs and vaping, QJM, Vol. 111, No. 3, 2018.*
7. *A.K. Breitbarth, J. Morgan, A.L. Jones, E-Cigarettes – An unintended illicit drug delivery system, Drug and Alcohol Dependence, 192, 2018.*
8. *C.F. Jorgensen, B.S. Rasmussen, K. Linnet, R. Thomsen, Emergence of semi-synthetic cannabinoids in cannabis seized in Eastern Denmark over a 6-years period, J. Forensic Sci., Sep 2024.*
9. *V. Sehdev, M. Cheung, A. Sharma and D. Obeysekera, The effects of Vitamin E acetate and its role in vaping: A systematic review, WJARR, 14(01), 2022, 487-495.*
10. *M. Lin, Z. Zhang, H. Hao, P. Xiang, J. Zhao, Rapid determination of etomidate and its structural anaolgues in e-liquid by probe electrospray ionisation quadrupole time-of-flight spectrometry, J. Pharm. Biomed. Anal., 256, 2025.*
11. *Increasing detection of etomidate and analogues on illicit drug markets is becoming a global concern*, UNODC LSS Portal, Mar 2025.

AFSN Member Institutes

Country/Region	No	Name of Member Institute (Total no. of Member Institutes = 84) (Total no.of countries = 19)
Bangladesh	1	National Forensic DNA Profiling Laboratory
Brunei Darussalam	2	Department of Scientific Services
Cambodia	3	Narcotic Laboratory of Secretariat General of National Authority for Combating Drugs
India	4	Centre for DNA Fingerprinting and Diagnostics
	5	Directorate of Forensic Science, Himachal Pradesh
	6	Forensic Science Laboratory, Govt. of NCT of Delhi
	7	National Forensic Sciences University, Delhi Campus
Indonesia	8	Indonesian Automated Fingerprint Identification System of the Indonesian National Police
	9	Department of Police Medicine - Centre for Medical and Health Sciences of the Indonesian National Police
	10	Eijkman Institute for Molecular Biology
	11	Forensic Laboratory Centre of Indonesian National Police Headquarters
	12	Indonesian Association of Forensic Pathologist
	13	Laboratory of National Narcotics Board Republic of Indonesia
	14	Master Program of Forensic Science, Postgraduate School, Universitas Airlangga
Lao PDR	15	Food and Drug Quality Control Center
Malaysia	16	CyberSecurity Malaysia
	17	Department of Chemistry
	18	Forensic Investigation Unit, Enforcement Division of Royal Malaysian Customs
	19	Malaysian Communications and Multimedia Commission
	20	Malaysian Institute of Road Safety Research (MIROS)
	21	National Institute of Forensic Medicine, Hospital Kuala Lumpur
	22	Pharmacy Enforcement Division, Ministry of Health, Malaysia
	23	Royal Malaysia Police Forensic Laboratory
Mongolia	24	Mongolian National Institute of Forensic Science
People's Republic of China	25	Beijing Forensic Science Institute
	26	Criminal Investigation School, Southwest University of Political Science and Law
	27	Department of Forensic Science (Department of Criminal Science and Technology), School of Investigation, People's Public Security University of China
	28	Drug Intelligence and Forensic Center of Ministry of Public Security
	29	Forensic Science Center of Guangdong Provincial Public Security Department
	30	Forensic Science Division, Department of Fujian Provincial Public Security
	31	Gansu University of Political Science and Law, Key Laboratory of Evidence Science Techniques Research and Application
	32	Guangzhou Forensic Science Institute
	33	Institute of Forensic Science, Fudan University
	34	Institute of Forensic Science, Ministry of Public Security
	35	Institute of Forensic Science, Dezhou Public Security Bureau
	36	Institute of Forensic Science, Hangzhou Public Security Department
	37	Institute of Forensic Science, Shanghai Municipal Bureau of Public Security
	38	Institute of Forensic Science, Shandong Public Security Department
	39	Institute of Forensic Science, Suzhou Public Security Bureau
	40	Institute of Forensic Science, Tianjin Public Security Bureau
	41	Nanjing Police University
	42	The Institute of Evidence Law and Forensic Science, China University of Political Science and Law
	43	Forensic Science Division of the Government Laboratory, Hong Kong Special Administrative Region
	44	Identification Bureau, Hong Kong Police Force, Hong Kong Special Administrative Region
	45	Forensic Science Department of Judiciary Police, Macau Special Administrative Region

AFSN Member Institutes

Country/Region	No	Name of Member Institute (Total no. of Member Institutes = 84) (Total no.of countries = 19)
Philippines	46	Laboratory Service, Philippine Drug Enforcement Agency
	47	National Bureau of Investigation
	48	National Reference Laboratory for Environmental, Occupational Health, Toxicology and Micronutrient Assay, East Avenue Medical Center, Department of Health
	49	Natural Sciences Research Institute, University of the Philippines Diliman Quezon City
	50	Philippine National Police
Republic of Kazakhstan	51	Forensic Examinations Centre of the Ministry of Justice
Republic of Uzbekistan	52	Republican Scientific and Practical Center of Forensic Medical Examination, Ministry of Health
	53	Republican Center for Forensic Examination under the Ministry of Justice
Singapore	54	Corrupt Practices Investigation Bureau
	55	Health Sciences Authority
	56	Ministry of Defence
	57	Ministry of Home Affairs
South Korea	58	Daejeon Health Institute of Technology, Daejeon Health Sciences University
	59	Department of Forensic Sciences, Sungkyunkwan University
	60	Graduate School of Forensic Science, Soon Chun Hyang University
	61	Institute of Forensic and Anthropological Science
	62	Korea Coast Guard Research Institute
	63	National Digital Forensic Center of Supreme Prosecutors' Office
	64	National Forensic Service
	65	Scientific Investigation Center of Korean National Police Agency
	66	Scientific Investigation Laboratory, Ministry of National Defense
	67	SJS Institute of Forensic Science & Medicine
Sri Lanka	68	Government Analyst's Department
	69	National Dangerous Drugs Control Board
Thailand	70	Central Institute of Forensic Science
	71	Central Police Forensic Science Division
	72	Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University
	73	Department of Forensic Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University
	74	Division of Forensic Medicine, Thammasat University Hospital
	75	Department of Medical Sciences
	76	Faculty of Medicine, Chiang Mai University
	77	Human Genetics Unit, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University
	78	Institute of Forensic Medicine, Police General Hospital, The Royal Thai Police
	79	Narcotics Analysis and Technical Service Institute Office of Narcotics Control Board
The Republic of the Union of Myanmar	80	Defence Services Medical Research Centre
Timor-Leste	81	Polícia Científica de Investigação Criminal - Laboratório de Polícia Científica
Vietnam	82	Forensic Medicine Center of Ho Chi Minh City
	83	National Institute of Forensic Medicine
	84	Vietnam Forensic Science Institute