ForensicAsia
THE ASIAN FORENSIC SCIENCES NETWORK NEWSLETTER | ISSUE 8 | 2018

AFSN President's Address

Dear colleagues and friends,

Time flies. AFSN has been ten years old since its founding in 2008. There are 51 member institutes from 15 countries as at 21 June 2018 and 6 workgroups (DNA, Trace Evidence, Crime Scene Investigation, Illicit Drugs, Toxicology, and Digital Forensic) and a Quality Assurance & Standards Committee. During the past 10 years, AFSN has already been the most important platform for forensic science technical communication, training, quality enhancing and strategy developing in Asia. It is all because of our members' devotion that makes AFSN better and better. I would like to show my appreciation to all colleagues and friends for their great contribution.

Forensic science has complex interaction with science, technologies and society. Indeed, forensic science is not only of vital importance for fighting against crime, but also serves for social governance. We are facing a fast changing world with new technologies continuously emerging and crime itself showing new form, more intelligent and border crossing. Forensic science needs to continue to develop to answer the new challenges, which means besides developing the new technologies, we also need to work together, to construct, to share and to form consensus. The annual meeting and newsletter just give us excellent opportunities to do so.

ForensicAsia not only act as a newsletter that records major events of AFSN, but also has provided a platform for Asian forensic experts to communicate progress in techniques and share opinions. I am so glad the 8th Issue is going to be published online. I would like to thank the editorial committee and all authors that shared their articles.

Annual meeting is a forum that gives us opportunity to invite experts from all over the world and have all members coming together to discuss how to support criminal justice system better. The 10th AFSN Annual Meeting & Symposium is going to be held in Beijing, hosted by my home institute, Institute of Forensic Science (IFS), Ministry of Public Security, China. I wish this meeting would mark the past progress and the new historical starting point for AFSN to have better and faster development.

A single flower does not make spring, while one hundred flowers in full blossom bring spring to the garden. I would like to work with all board members to play a more active role to achieve AFSN objectives and to enhance collaboration among different forensic institutes in countries within or outside Asia.

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

Content

Editor's Address

Dear colleagues and members of AFSN,

As the new Editor of the *ForensicAsia,* I am very pleased to see the enthusiasm of our members and the overwhelming response on the number of articles we received after the announcement to call for articles in March 2018 and to publish the 8th Issue of *ForensicAsia* this year. And for the first time, we do not print *ForensicAsia* in hardcopy, but is published online.

ForensicAsia has been an important newsletter that connects all our member institutes in the Asian Forensic Sciences Network (AFSN). Since the inception of AFSN in 2008, we have grown from 6 member institutes to 51 member institutes, across 15 countries in Asia over the last 10 years. Since then, *ForensicAsia* has been an integral part of AFSN and serves as a platform to announce news, share experience and promote collaborations in forensic science.

In this issue, we have a total of 9 technical articles and case studies, including, fires and explosions, forensic biology & DNA, illicit drugs, toxicology and general forensic science. In addition, we have also received contributions on AFSN news, international scene and introduction of our member institutes.

I would like to take this opportunity to thank all the authors who have contributed to the 8th Issue of *ForensicAsia*. I would also like to thank our editorial committee who spent their time in reviewing the articles, and our editorial assistants who help with the administrative matters and put together articles and artwork of this publication, to make it a success. Last but not least, I would like to thank our former Editor, Assoc Prof Christopher Syn, who had put in tremendous effort in advancing *ForensicAsia* to a new height.

Happy reading!

Dr Lui Chi Pang
Editor

Upcoming Events

Date	Event Name
4 Sep – 8 Sep 2018	AFSN 10th Annual Meeting and Symposium, Beijing, People's Republic of China
9 Sep – 13 Sep 2018	The Australian and New Zealand Forensic Science Society (ANZFSS) 24th International Symposium, Perth, Western Australia
24 Sep – 27 Sep 2018	International Symposium on Human Identification (ISHI), Phoenix, Arizona, United States of America
9 Oct – 12 Oct 2018	17th Annual Association of Forensic Quality Assurance Managers (AFQAM) Training Conference, Providence, RI , United States of America
7 Oct – 12 Oct 2018	Society of Forensic Toxicology, Minneapolis, MN, United States of America
18 Feb – 23 Feb 2019	American Academy of Forensic Science (AAFS) 71st Annual Scientific Meeting, Baltimore, MD, United States of America

The 8th AFSN Annual Meeting and Symposium 2016

Pol. Lt. Col Ampika Leeapojanaporn
Central Institute of Forensic Science, Thailand

The 8th AFSN meeting "Towards Forensic Standards in Asia: Integration, Unity, Collaboration" was held at the Berkley Hotel Pratunam, Bangkok from 16 to 19 August 2016. There were 517 attendees, from 40 member institutes in 13 countries. There were 13 invited speakers, from all over the world including Australia, America, Europe, and of course, Asia. During the breakout sessions, there were 54 oral presentations and 44 posters from AFSN members.

This was the first AFSN Symposium where the legal aspects and jurisprudence of science were highlighted and discussed. This allowed the delegates to understand how their scientific work is required to be of a very high standard so that legal decisions can be reliably based on their work.

Just as the plenary sessions covering important and diverse subjects, such as, the discussion on Forensic science for jurisprudence, Jurisprudence: Forensic science and ASEAN community and Forensic research & education. Another important aspect of the meeting was to begin the process of developing global forensic networks.

Each individual workgroup held a workshop. The DNA Workgroup studied the human rights aspects of DNA analysis and uncertainty of measurement and worked hard to write standards relevant to the Asian forensic scene. The Crime Scene Investigation Workshop studied Bomb scene investigation, and interpretation of bomb debris. The Illicit Drugs Workshop studied the SWGDRUG recommendations and NPS trends with the Toxicology Workshop concentrated on systematic drug analysis in body fluids. The Trace Evidence Workshop looked at microanalysis in trace evidence. The Quality Assurance and Standards Committee Workshop also studied the SWGDRUG recommendations as well as the design of a training program for performance.

Apart from working hard, the delegates were able to enjoy a full social program including a welcome dinner where many contacts and friendships were created and reinforced.

The 9th AFSN Annual Meeting and Symposium 2017

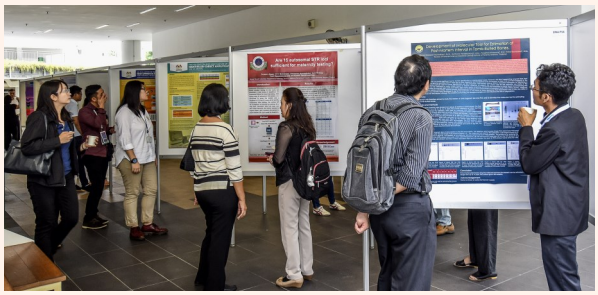
Ms. Joyce Heng
Health Sciences Authority, Singapore

Together with the Asian Forensic Sciences Network (AFSN), HSA organised the 9th AFSN Annual Meeting and Symposium themed, "Advancing Forensic Science in a Rapidly Changing World" at the Ngee Ann Polytechnic, who is also our local co-organizer for the event.

More than 600 overseas and local participants from 21 countries and 83 institutes attended the event held from 5 - 8 September 2017, with another day of pre-conference activities on 4 September 2017.

We were privileged to have Mr Desmond Lee, Minister for Social and Family Development and Second Minister for National Development to grace the Opening Ceremony on 5 September 2017. We also had many other VIPs from State Courts, AGC, MHA, CNB and SPF/CID.

Over the five-day event, participants gained valuable knowledge from the various insightful lectures from 19 renowned speakers and experts, 81 oral and 54 posters presentations, diverse disciplines of workgroup sessions and on-site forensic facility visits.



The event was supported by 15 sponsors who put up impressive booth displays and interesting games for participants.

Guest-of-Honour Mr Desmond Lee with CEO of HSA, Principal of Ngee Ann Polytechnic, AFSN President and Board members and Keynote Speakers.

Participants also had the opportunity to visit the facilities of CID/SPF, CNB, INTERPOL and HSA laboratories on 8 September 2017.

On 7 September 2017, about 200 participants and guests enjoyed a conference dinner at the Forest Lodge, Singapore Zoo, followed by a guided tram ride at the Night Safari.

AFSN Board members at the AFSN Annual General Meeting, which was held on 8 September 2017.

The International Criminal Court, Office of the Prosecutor Scientific Advisory Board

Dr. Linzi Wilson-Wilde OAM
National Institute of Forensic Science Australia New Zealand (ANZPAA)
International Forensic Strategic Alliance (IFSA) Representative

The 5th meeting of the International Criminal Court (ICC) Office of the Prosecutor Scientific Advisory Board (OTP SAB) was held 21-22 June 2018 in The Hague, Netherlands.

The ICC was established following the adoption by 120 states of the Rome Statute in 1998 and subsequent ratification by 60 states in 2002. The Rome Statue is the legal and governing document for the ICC and member states ratify the Statue in order to become members of the ICC; there are currently 123 member states [1].

The ICC is based in The Hague, Netherlands and investigates, and where appropriate tries, individuals for serious crimes to the international community: genocide, war crimes, crimes of aggression and crimes against humanity. To do this the ICC has over 900 staff organized into one of four organs of the court: Presidency, Judicial Divisions, OTP and Registry [1].

To date there have been 26 cases before the Court, with some cases having more than one suspect. ICC judges have issued 32 arrest warrants, nine people have been detained in the ICC detention centre and have appeared before the Court and 15 people remain at large. Charges have been dropped against three people due to their deaths. ICC judges have also issued nine summonses to appear [1].

The judges have issued verdicts in six cases: nine people have been convicted and one acquitted. There are currently three cases before the court: the Ongwen case, the Ntaganda case, and the Gbagbo and Blé Goudé case. Further details can be found on the ICC website. [1].

The OTP SAB was established in 2014 and provides recommendations to the ICC Prosecutor. Recommendations are provided in relation to the collection, management and analysis of scientific evidence relating to the investigation and prosecution of genocide, crimes against humanity and war crimes. The goal of the OTP SAB is to assist in adapting the Office's investigative and prosecutorial capabilities and networks to the rapidly changing scientific and technological environment and meet the strategic goal set by the Prosecutor for the Office [2]. The OTP SAB comprises representatives from 18 international networks and organisations from all world continents.

Areas of interest to the OTP and the SAB relate to forensic evidence that can show patterns of displacement (e.g. seismology) and digital evidence. Other forensic disciplines that have been important in casework include imaging, document examination, DNA, anthropology, crime scene examination and pathology/exhumations. A robust technique for age determination of minors would also be important.

References

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Deputy Prosecutor, Mr James Stewart with the members of the Office of the Prosecutor Scientific Advisory Board, June 2017.

The Office of the Prosecutor Scientific Advisory Board in session, June 2017

Cutting drugs with new highly toxic adulterants in China

Dr. Xiaoyu Zheng, Mr. Yanbiao Zhao, Ms. Hongxian Yang, Ms. Hui Zheng, and Mr. Lisheng Gao
Institute of Forensic Science, Ministry of Public Security, China

Introduction

Cutting agents including diluents and adulterants are intentionally added to illicit drugs mainly to increase the profits, to give the impression of a better quality drug or mask a poor quality drug using substances with similar properties. Although they are present in the majority of the seized drugs exhibits, they are frequently not reported either because they are non-controlled, they weren't identified or they may be removed in the extraction. Colombo Plan uncovered an unprecedented trend of cutting drugs along the entire supply chain with new highly toxic adulterants over the past years. According to the summary of the 1st International Symposium of Forensic Drug Testing Lab Directors held in Mexico, the cutting agents are quite different by countries even by provinces or states and in order to provide a global early warning system for the emergence of new toxic adulterants, an international database of cutting agents should be developed. Herein, this study on the trend of cutting drugs in China may serve as a reminder to all the countries those fail to identify the toxic adulterants and can impede the identification of underlying causes for national-level drug epidemics as well.

Materials and Method

Extraction

A seized material named "happy water" was used in the analysis. Two extraction methods were applied in this study.

1) The liquid was vaporized to dryness and appropriate amount of methanol was added before directly examined by GC/MS.

2) Liquid-liquid extraction process. The sample was adjusted to a pH value of 13 with NaOH and then extracted with $CHCl_3$. The corresponding spectra were shown in Figure 1(a) and (b), respectively.

Instrument condition

Agilent 6890N GC coupled with 5975B mass–selective detector with column DB-5 MS 30 m × 0.25 mm × 0.25 μm, temperature: programme 60ºC (0 min) with 15ºC/min to 300ºC (15 min); injector: 280ºC; split ratio: 20:1; transfer line: 250ºC; ion source: 230ºC; ionization energy: 70 eV; injection volume: 1 μL; Helium gas with flow: 1.0 mL/min; Scan parameter: 40-500 m/z.

Results and Discussion

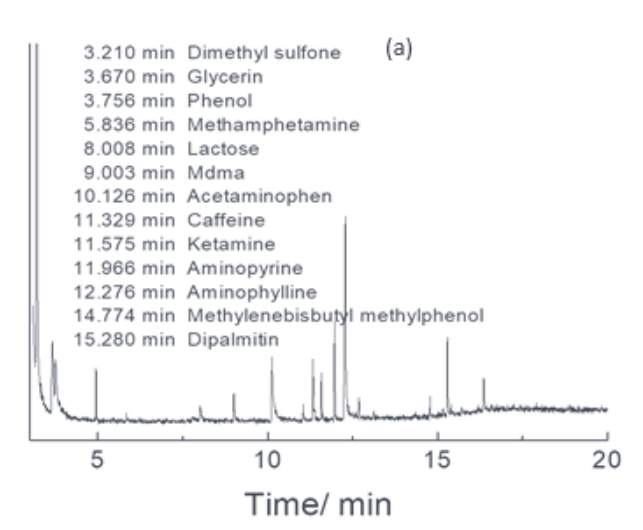

Figure 1: Spectra of "happy water" analyzed by GC/MS. (a) Directly analyzed. (b) Analyzed after liquid-liquid extraction.

Figure 1 shows some of the substances including glycerin, phenol, lactose, acetaminophen, aminophylline and dipalmitin were removed in the extraction method. Results from data also have indicated that plenty of drugs are increasingly cut with multiple and highly toxic adulterants. In fact, the cutting agents have been more and more highly toxic and have caused serious public health implications all over the world. The toxic adulterants and impurities, alone or in combination, can cause poisonous medical effects, including death[1, 2].

To gain a more comprehensive understanding of trend of cutting drugs in China, the seized samples or street drugs were analyzed with GC-MS, LC-IT-TOF, and HTRM. From the results of 1563 cases analyzed in our lab in the last year dimethyl sulfone, caffeine, and N -isopropyl benzylamine are supposed to be the most commonly used cutting agents in China as shown in Figure 2, this result is attributed from high number of seized methamphetamine (911 cases). Furthermore the results also shows that the most prevalent adulterants are different by drugs. Heroin (285 cases) is mostly cut with caffeine (35.1%), acetaminophen (23.8%) and procaine (8.4%), while cocaine (63 cases) is mostly cut with caffeine (20.6%) and levamisole (19.0%).

Conclusion

This study on toxic adulterants in drugs will not only greatly assist law enforcement in accomplishing the missions but also be useful for criminal investigation and monitoring routes of trafficking. For prevention and information purposes, it would be very meaningful for all the countries to strengthen research on the toxicological properties and the health impacts of cutting agents. An international standardized drug testing methods should be developed. Further studies on biological specimen such as urine, blood, oral fluid and hair should be carried out in order to monitor the trend of the new toxic cutting agents.

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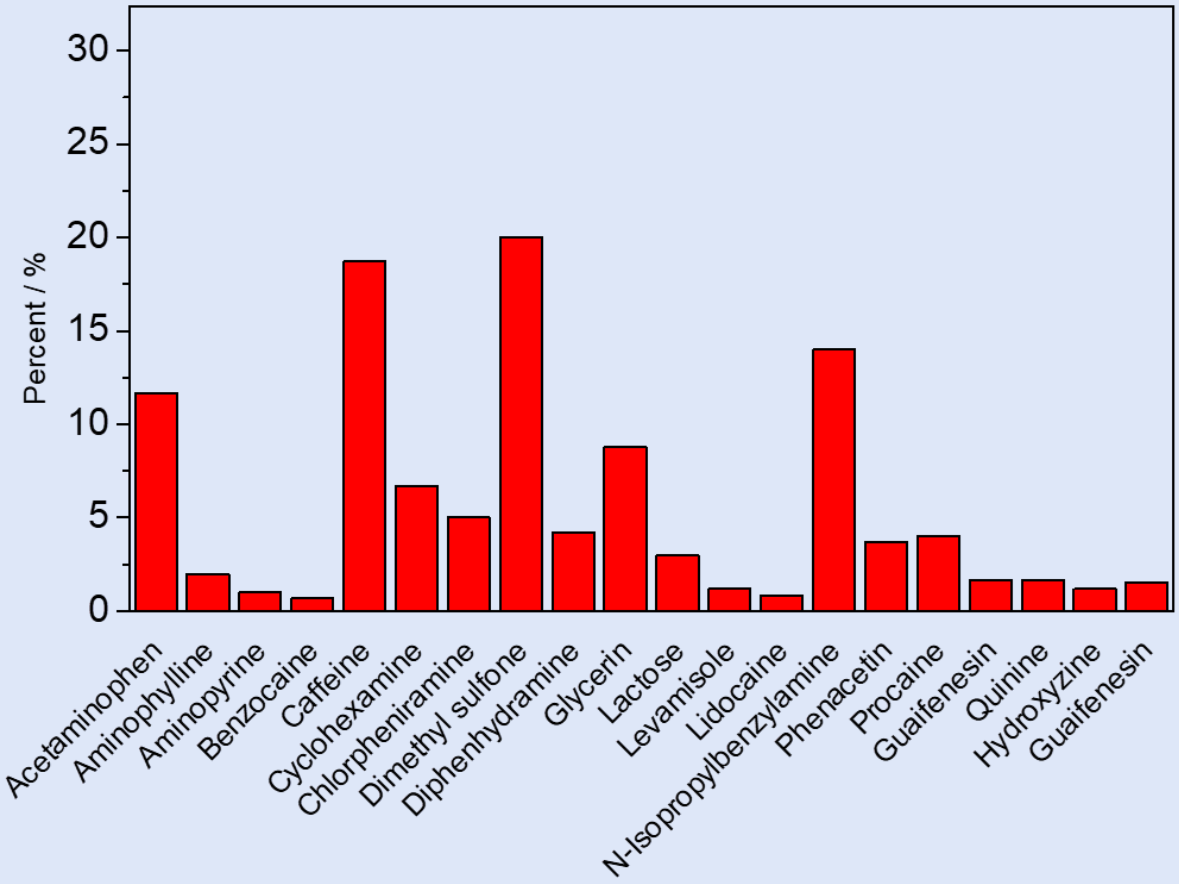

Figure 2: Cutting agents in seized materials in 1563 cases

Firearms/Toolmarks Analysis: Moving Forward as a Community

Dr. Alaric C W Koh, Ms. Lim Shing Min
Health Sciences Authority, Singapore

One recommendation of the United States' President's Council of Advisors on Science and Technology (PCAST) report issued in 2016 [1] was the development of objective methods for firearms/ toolmarks analysis. Aided by advances in imaging techniques, there has been rapid progress in the application of statistical and machine learning methods in recent years, and the interested reader is referred to [2-6] for some examples.

Another recommendation made by PCAST was to improve "firearms analysis as a subjective method" through having more studies to further establish its scientific validity, notwithstanding the numerous studies [7-10] that were already published up to that point. There have been more published studies since the PCAST report, including an isolated pairs study [11], a blind test [12], and one using virtual microscopy [13].

The Forensic Chemistry and Physics Laboratory, Health Sciences Authority, is also sponsoring a study to determine the error rates associated with the examination of toolmarks left by ten consecutively manufactured snap-off blades in puncturing plastic tubes. As of June 2018, we have received results of over 30 participants from 9 countries. The study is still open, and interested toolmark examiners of AFSN member institutes are invited to contact the authors (alaric_koh@hsa.gov.sg / lim_shing_min@hsa.gov.sg) or apply through this link [14].

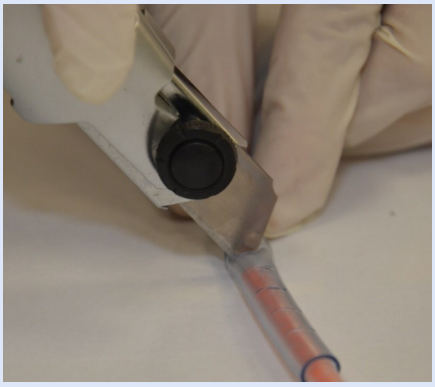

Figure 1: The author puncturing a plastic tube using one of the snap-off blades.

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Korea Coast Guard Research Center, Republic of Korea

Mr. Han Seong Lee
Korea Coast Guard Research Center, Republic of Korea

Introduction

Korea Coast Guard (National Maritime Police Agency) was established as an agency belonging to the National Police Agency for law enforcement against illegal fishing boats and the maintenance of maritime security in 1953. As the Ministry of Oceans and Fisheries was launched in 1996, it has expanded to the Korea Coast Guard (National Maritime Police Agency) to this day. Currently, the Korea Coast Guard have about 300 patrol vessels and aviation power of 30 aircraft, and about 12,000 people protecting the sea. Korea Coast Guard guards the Exclusive Economic Zone (EEZ) and performs tasks, such as maintaining public order at the sea, coping with marine accidents, controlling maritime pollution and establishing the maritime traffic order.

Organization Structure

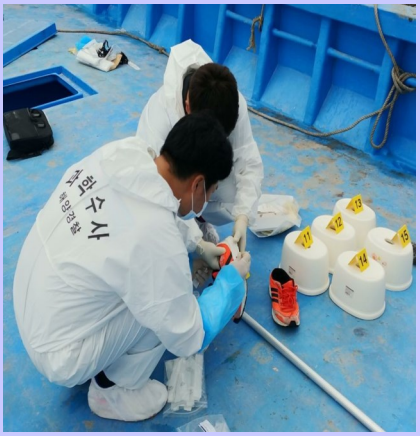
Korea Coast Guard Research Center started to detect contaminants in the ocean in charge of testing and research belonging to the Marine Pollution Control Officer in August 1978. As the scope of tasks has expanded now, it conducts research and development of a variety of equipment and countermeasure technology for the Korea Coast Guard's promotion of tasks and has grown up as an institution that develops and appraises Forensic Science technology specializing in the ocean. Korea Coast Guard Research Center consists of the Research Planning Team, Marine Pollution Response Research Team, Korea Coast Guard Research Team, Forensic Science Team and Identification and Analysis Team. On the sea, unlike on land, it is impossible to preserve the scene which is influenced by water temperature/tidal current/ebb and flow/depth of the water/salt. It is hard to find evidence which can be damaged by the sea. Thus, it operates Forensic Science Team and Identification and Analysis Team to develop Forensic Science technology specializing in the ocean.

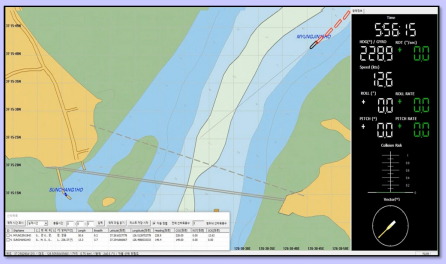
Team Name	Tasks
Forensic Science Team	DNA profiling and Digital forensics-related research
Identification and Analysis Team	Trace evidence analysis, Oil spill identification and efficiency test of materials in oil spill response
Research Planning Team	Administration, asset management, personnel, finance, government administration
Marine Pollution Response Research Team	Development of response equipment and technology for counteracting marine pollution
Korea Coast Guard Research Team	Development of equipment and technology for maritime security and rescue

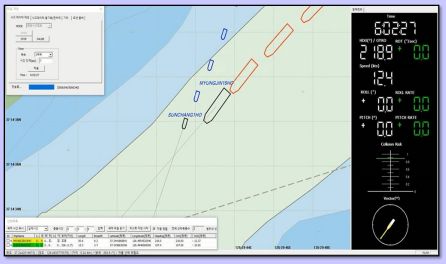
Forensic Science Team

The Forensic Science Team conducts digital forensics and DNA profiling. It reproduces ship collision and proves the illegal charges, restoring data from a variety of digital navigation equipment. It reveals identification from unknown dead bodies on the sea and the evidence of charge with DNA profiling, supports DNA profiling for the crackdown of illegal whale meat distribution and is the only institution in

Korea, which has the original technology that can do the DNA profiling from the flooded evidence in the ocean. Especially, it investigates the techniques for drowning location and time estimation to solve the cases of unknown dead bodies on the sea as soon as possible.



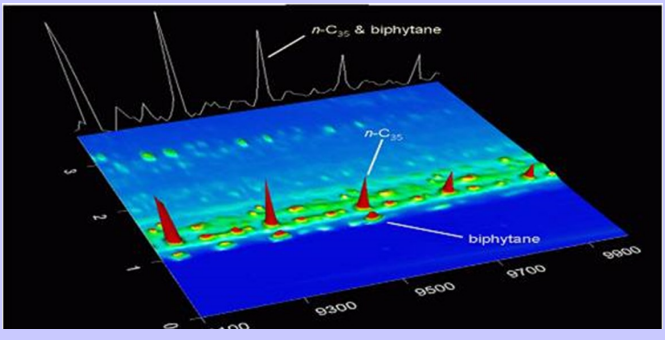


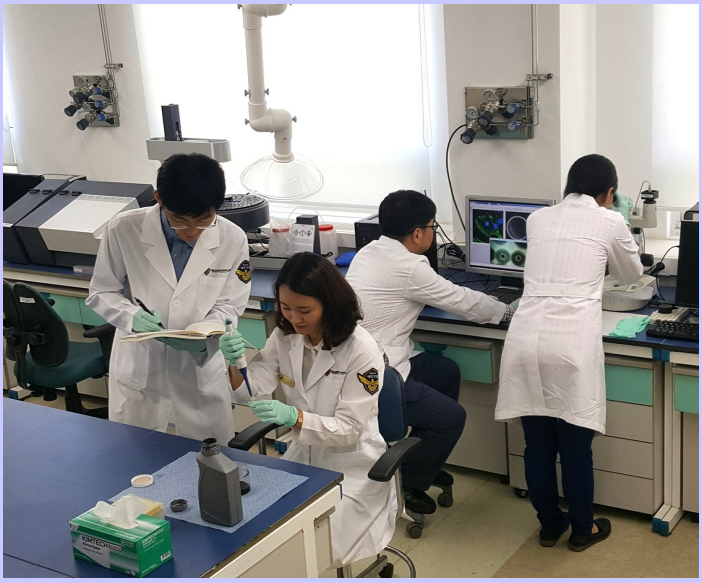


Identification and Analysis Team

The Identification and Analysis Team analyzes paint trace of ship collision, falsification of tax-free oil, oil fingerprint analysis of illegal oil discharge in the sea and then efficiency test of materials such as dispersants and sorbents in oil spill response. Especially, trace evidence and oil spill identification by chemical fingerprint are powerful forensic tools. Moreover, it analyzes for management Fuel oil and lubricants used in vessels of Korea Coast Guard.

Korea Coast Guard Research Center is the only institution in Korea to investigate Forensic Science specialized in the sea and to make appraisals and makes a constant effort to realize the clean and safe sea with Forensic Science specialized in the sea.





Korea Coast Guard Research Center / Forensic Science Research Team
Kim Nam Yul
Tel. +82-41-640-2681, +82-10-5663-1129
Fax. +82-41-640-2832
E-mail. sgryul1129@gmail.com

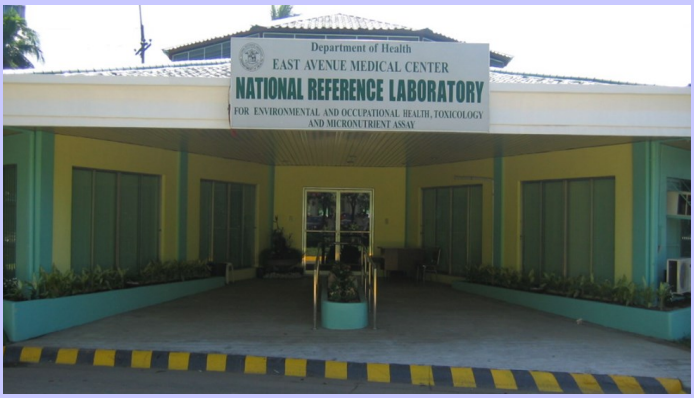
The Philippine National Reference Laboratory for Environmental and Occupational Health Toxicology and Micronutrient Assay

Mrs. Nenita G. Marayag, RCh, MPA; Ms. Anna Katrina Tadeo, RCh
National Reference Laboratory for Environmental and Occupational Health Toxicology and Micronutrient Assay (NRL-EOHTM), Department of Health, Philippines

Introduction

The National Reference Laboratory for Environmental and Occupational Health, Toxicology and Micronutrient Assay or NRL-EOHTM is under the Department of Health and mandated to perform the following functions:

- Provides laboratory reference/referral services for confirmatory testing, surveillance and research
- Trains laboratory personnel
- Maintains quality assurance program for laboratory tests through proficiency testing
- Performs technical evaluation of reagents and diagnostic kits.



Accreditation

The NRL-EOHTM is ISO/IEC 17025:2005 accredited for water (chemical and microbiological) and drug (screening and confirmatory) testing as well for toxicology analysis. The goal of the NRL at East Avenue Medical Center is to provide leadership and technical expertise at the national level for laboratory services in the field of environmental, occupational health, toxicology and micronutrient assay. NRL is the only laboratory under the Department of Health (DOH) that provides laboratory services for higher levels of water analysis, drug testing and toxicology.

Pursuant to the implementation of Quality Assurance Program, NRL-EOHTM is responsible for the domestic proficiency tests for drug and water laboratories all over the country. The proficiency test (PT) samples are distributed once a year to over 1200 laboratories for screening and 140 laboratories for water microbiological testing. The PT materials used both for water and drug testing are all prepared in-house by NRL following ISO/IEC 17043. Laboratories who have failed to meet satisfactory results, their licenses to operate are revoked or suspended.

"NRL-EOHTM provides technical trainings for competent, compassionate, ethical and socially responsive laboratory workers nationwide. Different trainings/ workshops for Medical Technologists, Chemists, Pharmacists and Doctors of Medicine are being conducted at different locations, regions and provinces in the country using various methodologies e.g. lectures, hands-on activities, interactive discussion and exams combined. This includes laboratory management, sampling and analysis with its laws, policies and regulations in the field of toxicology, environmental and occupational health."

The Department of Health, with the Food and Drug Administration (FDA-Philippines) in pursuit to protect public health, has also tasked the reference laboratory with the evaluation of products as a requirement on the issuance of Certificate of Product Registration (CPR). NRL conducts performance evaluation for screen drug testing using kits or automated machine as well as water treatment devices, water test kits and health care wastes treatment devices.

NRL-EOHTM is investing on establishing laboratory services in providing the highest level of diagnostic technology, surveillance and monitoring. This includes improving further the quality of services to be delivered to the clients through employing highly technical skilled laboratory staff, expanding capability,

developing new methods of testing using different types of environmental and biological sample in preparation to face challenges brought by emerging problems on drugs of abuse, environmental and occupational health.

Moreover, NRL-EOHTM participates in the formulation of standards and policies to recommend best practices in the laboratory as implemented by regulatory agencies. It provides technical support to the programs of the Department of Health related to the fields to ensure availability of the required analysis and to offer scientific support assisting government initiatives, activities and programs.

Government Analyst's Department of Sri Lanka Today

Mr. Ariyananda Welianga
Government Analyst's Department, Sri Lanka

Introduction

Established in the year 1904, the Government Analyst's Department (GAD) is the Central Government Laboratory in Sri Lanka under the purview of the Ministry of Justice. Government Analyst's Department is a member institute of the Asian Forensic Sciences Network since 2016.

Vision of the Department is ***to Form a Fair and Law Abiding Society.*** Mission is ***Providing Analytical, Advisory and Consultancy Services in the Fields of Forensic and Food Science to Law Courts and Law Enforcement Agencies and other Institutions.***

Organization Structure

Government Analyst's Department is the only Forensic Science Laboratory in the country and it consists of two major divisions as Forensic Science and Food Science. The Forensic Science Division is the sole provider of forensic analytical and advisory services to Law enforcement agencies in the country, is fast becoming an increasingly prominent forensic service provider. Experts of the Government Analyst's Department appear as expert witnesses and testify in the Courts of Law in the country.

There are ten individual laboratories accommodated in six storied building complex with an indoor firing range in the basement for test-firing of guns and two vehicle lifting points for vehicle examinations. Laboratories are DNA, Digital Forensic, Narcotic, Toxicology, Firearms, Explosives/Fire, Miscellaneous, Questioned Document, Food and Liquor.

Accreditation

Government Analyst's Department which is accredited by the Sri Lanka Accreditation Board (SLAB) meets all requirements of ISO/IEC 17025:2005, demonstrating technical competence and operation of a laboratory Quality Management System (QMS) as a testing Laboratory under the field of Chemical and Forensic Testing.

Digital Forensic Laboratory

Government Analyst's Department, having most of the investigation facilities, equipment and knowledge pertaining to various crime investigations, facilities to analyze digital crimes has been a long-felt need for the country. In the year 2015 fully equipped Digital Forensic Laboratory with all the equipment including training was donated by the Government and the people of the Republic of Korea through Korea International Corporation Agency (KOICA).

DNA Laboratory

DNA laboratory of the Government Analyst's Department was established in 2013 with the aid of USAID (United States Agency for International Development) and it was the first Government DNA Laboratory in the country. It was further strengthen by providing equipment, training and chemicals through the KOICA project in 2015. DNA analysis of bones, teeth and Mitochondrial DNA analysis play a major role in crime investigation and has been also developed under the KOICA project thereby the laboratory is now capable of analyzing all type of forensic DNA samples.

Being provided with the modern high technological equipment and training of officers of DNA Laboratory and Digital Forensic Laboratory by the National Forensic Services (NFS) of Ministry of Government Administration and Home Affairs, Republic of Korea through KOICA project, the Government Analyst's Department is a national asset to Sri Lanka.

The above project was launched with excellent coordination of the former Director General of the Government Analyst's Department, Government Analyst, Mrs. Sakunthala Tennakoon and also with great assistance of the former Director General of NFS, Korea, Dr. Seo Joong Seok.

Government Analyst's Department,
31, Pelawatta, Battaramulla,
Sri Jayewardenepura,
Sri Lanka

Timor-Leste Police Scientific Laboratory and Criminal Investigation; Its Challenges and Opportunities

Mrs. Monica Alda Guterres Menezes
The Scientific Police and Criminal Investigation, Timor-Leste

Introduction

The Scientific Police and Criminal Investigation (PCIC) of Timor-Leste is a superior criminal-police institution with its mission to support justice system in Timor Leste by conducting a criminal investigation with a more complex process.

The institution was established under Ministry of Justice with autonomy both administration, financially and patrimonial. Even though PCIC has autonomy and independence under Ministry of Justice, PCIC also has responsibility to follow public prosecutor regulation and the public prosecutor to focalize work of PCIC.

The PCIC has a main scientific principal to work with and support the Police Scientific Laboratory (LPC) in order to carry out a criminal investigation and to guarantee evidence is well collected, examined, and stored with full responsibility.

The institution is well equipped to investigate crimes committed by organized, modern and sophisticated criminal groups. This type of criminal activity has become a serious challenge in the context of socio-economic development. It, therefore, requires an independent scientific-police-institution that is technically well-prepared with the support of scientific laboratory to support the institution when conducting a criminal investigation.

Challenges and Opportunities

Human resources

Currently, LPC has 30 officers; 4 Scientists and 26 laboratory technicians. The mentioned Scientist is from different backgrounds, skills, and ability; 16 officers specialized in the criminalist, 4 are in toxicology, 5 are in document fraud and another 5 are in ballistics. These officers were trained in School of Judicial Police-Portugal for 3 months in general area of Criminalities, Toxicology, Document falsification and Ballistics.

Laboratory equipment

As the newest institution, PCIC has laboratory materials to support its scientists and laboratory officers to collect crime evidence from crime scene and perform laboratory analysis. There are two mobile laboratories with full equipment to conduct an investigation at the crime scene. These two mobile will then transport any evidence taken from the crime scene to the laboratory situated with the Police Scientific Laboratory (LPC) for further examination as they have more advance equipment such as VSC8000 which analyse documents forgery and counterfeit money. In addition to that LPC also have a microscope for analysing ballistics and its scene.

LPC support is to Back-Up investigation process

Since established in 2015, LPC has responded several requests from the Public Prosecutor office and court by conducting DNA test, lofoscopic, crime scene sketch, photograph scene, fire scene investigation and explosion.

LPC also has experience to carry out counterfeit money analysis, forgery, identify and analyse fingerprints, examine fire bullet and drugs. As the procedures, all results of examination, investigation and analysis are reported to relevant institution for further action.

However, PCIC – LPC still has limited resources, both human resources and equipment to do DNA, forgery and narcotic laboratory analysis. Therefore, LPC has established cooperation with Judicial Police of Portugal laboratory to support the analysis of DNA, forgery, and narcotics by sending sample of evidence to Judicial Police of Portugal laboratory to do examination as needed.

During 3 years of work, LPC has responded to several cases and requests as in the following table:

SECTOR	2015	2016	2017
Crime Scene	18	35	24
Judicial Identification	42	128	132
Lofoscopic	6	2	6
Forging and Counterfeit Money	9	15	8
Toxicology	10	10	7
Ballistics	-	1	2
DNA	4	3	5
Autograph	-	1	4
TOTAL	89	195	188

National Institute of Forensic Medicine, Viet Nam

Dr. Nguyen Duc Nhu
National Institute of Forensic Medicine, Viet Nam

Introduction

In Vietnam, the Forensic medicine system belongs to Vietnam Ministry of Health is organized in two levels. In central level: Vietnam National Institute of Forensic medicine; in provincial level: 63 Centers of Forensic medicine in 63 provinces and cities.

The National Institute of Forensic Medicine (NIFM) was founded on 17 January 2001 by the Decision of Prime Minister, Vietnam Government.

Organization and structure

NIFM is divided into 7 administrative, assisted departments and 7 professional departments and laboratories which have more than 50 experts in a variety of disciplines to perform professional work, including: medical doctors, pharmacists, chemists, biologists, and others. NIFM belongs directly to the Ministry of Health and NIFM has a Sub-institute in HoChiMinh city. The 7 professional departments/labs include:

1. Forensic medicine department
2. Forensic pathology department
3. Forensic DNA laboratory
4. Forensic toxicology laboratory
5. Forensic imaging department
6. Forensic toxicology laboratory in HoChiMinh city
7. Forensic medicine department in HoChiMinh city

Tasks and Responsibilities

- Providing forensic medicine services: Clinical forensic medicine examination (evaluating the health impairment and degree of injury, examining victims of sexual assault, age estimation); Forensic autopsy; Toxicology; Pathology and DNA.
- Research: Scientific researches on forensic medicine.
- Training: Organizing training and re-training courses for medical examiners across the country. Collaborating with medical universities for post-graduated training on forensic medicine.
- Guiding the activities of forensic medicine across the country (63 centers of forensic medicine in provinces and cities).
- International co-operation.
- Other functions according to the Decision of the Prime Minister such as providing immediate response in disaster situations.

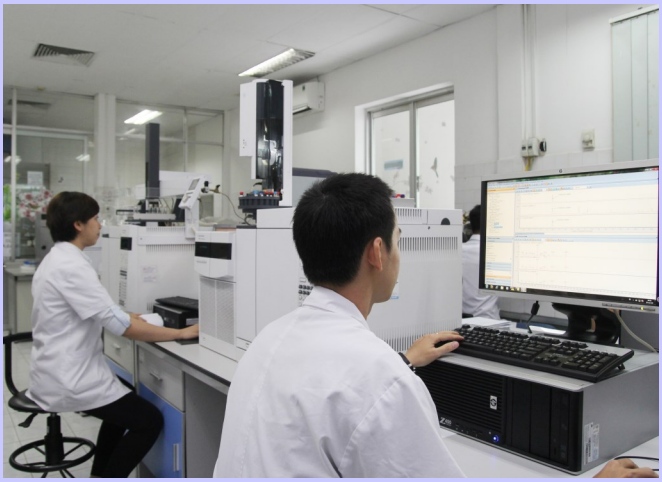
Services

1. Forensic medicine examination
   The forensic medical professionals examine individuals (victims, suspects) who are related to criminal cases and conduct injury or wound assessments. They also obtain biological evidence from these individuals for laboratory testing and medical opinion reporting.
   - Investigation of the cause and mechanism of death (dealing with such questions as who is the victim, where and when did the death occur, what types of trauma and damage were sustained by the victim, the mechanism and cause of death).
   - Identification of bodies and unidentified persons.
   - Examination of bodies at the scene where they are found.
   - Clinical examination, age estimation, assessment of damage and injury to victims of sexual and physical assault.
   - Examination of sexual or physical violence suspects to identify signs of trauma.
   - Assessment of the biological age of deceased or alive persons.
   - Provision of an opinion based on medical documents regarding death, or regarding damage from an injury in alive persons.

2. Forensic DNA Test
   - Using newest and probably most powerful DNA technology to identify the DNA evidence from varying samples as blood, hair, nails, bones, saliva, semen, samples in the scene such as clothes, cigarette butts and so on. The unit has recruited several forensic scientists who are competent in the procedure and protocols for DNA profiling as well as expert interpretation.
   - Assistance in the investigation of serious crime such as murder by DNA test.
   - Paternity testing under the request of an organization or individual order.

3. Forensic Toxicology
   The forensic toxicologists are responsible for the detection and quantitation of poisons in tissues, blood, urine, gastric content or any other body fluids obtained during autopsy. Their scientific findings will help in determining the cause of death or any other criminal cases.

4. Forensic Pathology
   The forensic pathologists conduct postmortem examination. The tissue and organ samples obtained during autopsy are analyzed in the pathological laboratory.



Collaborations

NIFM has close contacted with National Forensic Services in South Korea in exchange of knowledge and experience in difference forensic fields. We also have close contact with Argentine Forensic Anthropology Team (EAAF) and International Committee of Red Cross (ICRC) for training our staffs in Forensic Anthropology. In 2012, we became a member of Asia Pacific Medico-Legal Agencies (APMLA). In 2016, we were proud to become an official member of the Asian Forensic Sciences Network (AFSN). We continue seeking frequent exchange of knowledge and experience as well as collaborations with experts from other member institutes.

National Institute of Forensic Medicine (NIFM)
41 Nguyen Dinh Chieu street, Hai Ba Trung district, Ha Noi city, Vietnam.
www.nifm.org.vn.
Tel: (+84) 24.39746190
Fax: (+84) 24.39746187

Study on Matrix Interference in Pyrolysis Products of Polyvinyl Chloride

Mr. Cheng Fangbin[1], Mrs. Zhou Hong[2], Mr. Wan Chaochao[1], Dr. Sun Zhenwen[2], Mr. Liu Yao[1,2]**

[1.] People's Public Security University of China，People's Republic of China

[2.] Institute of Forensic Science, Ministry of Public Security，People's Republic of China

Abstract

In the detection process of arson cases, the identification of ignitable liquid debris is of great significance in determining the nature of the case and verifying the facts of the crime. Under the action of high temperature, polyvinyl chloride undergoes chemical reactions such as side group elimination and cyclization reaction, resulting in a large number of substances similar to the target characteristic components of ignitable liquids, which interfere with the data analysis and interpretation. In this paper, polyvinyl chloride was used as an experimental sample to study its pyrolysis behavior under different conditions such as different temperatures and heating times, and its background and pyrolysis interference components at high temperatures were characterized.

Introduction

Polyvinyl chloride (PVC) was once the world's largest production of general-purpose plastics, and widely used in daily necessities, industrial products, floor leather, pipelines, etc. In the fire field, some common polymers generally produce only a small amount of aromatic compounds, and have limited interference with the identification of ignitable liquid debris (ILRs)[1]. However, under the action of high temperature, PVC generates a large number of aromatic compounds, which seriously affect the identification of ILRs[2].

Materials and Methods

Chemicals and instrumentation

PVC was of analytical grade (Sinopharm Chemical Reagent Co., Ltd), far-infrared closed electric furnace (FL-2Y, Lichen Technology, China), infrared thermometer (DT-8833, Huashengchang, China), 20ml headspace bottle (C4020-2, Thermo Scientific, USA), 20mm header cap (C4020-36A, Thermo Scientific, USA), 100μm polydimethylsiloxane solid phase microextraction device (57300-U, SUPELCO, USA), heating block (H2050-1, Lab-Line, USA)

Blank experiment

About PVC (0.1g) was directly placed in a headspace vial and adsorbed on solid phase microextraction (SPME) for 30 min. And GC/MS was used to collect data.

100°C, 150°C heating experiment

Put about PVC (0.1g) into a headspace bottle and transfer to a heating block that has been heated to 100°C and 150°C respectively. After heating for 30 min, SPME is adsorbed for 30 min. GC/MS was used to collect data.

300°C, 500°C, 700°C heating experiment

About PVC (0.1g) was placed in the electric furnace that has been heated to 300°C, 500°C, 700°C, and then heat 15min, 3min, 0.5min, respectively. SPME is adsorbed for 30min. GC/MS was used to collect data.

GC/MS condition:

Column: J&W DB-5MS quartz capillary column (30m×0.25mm×0.25μm)
Carrier gas: helium, purity ≥99.995%
Column flow rate: 1mL/min
Column temperature: 40°C for 2min, 5°C/min to 80°C, 10°C/min to 280°C, 5min
Inlet temperature: 250°C
Transmission line temperature: 230°C
Injection method: split injection
Ion source: Electron bombardment source (EI)
Scan range: 40 amu to 400 amu

Results and Discussion

PVC pyrolysis mechanism

At high temperatures, PVC undergoes side group elimination, cyclization chemical reactions, resulting in a large number of aromatic compounds. In PVC and other compounds, the side group atoms or functional groups linked to C-C are more active. When subjected to high temperature, the side groups will first be cleaved to form small molecules. When the side group elimination reaction reaches a certain degree, a series of conjugated double bonds are formed in the C-C main chain and subsequent cyclization of the unsaturated chain occurs, resulting in a large amount of aromatic compounds.

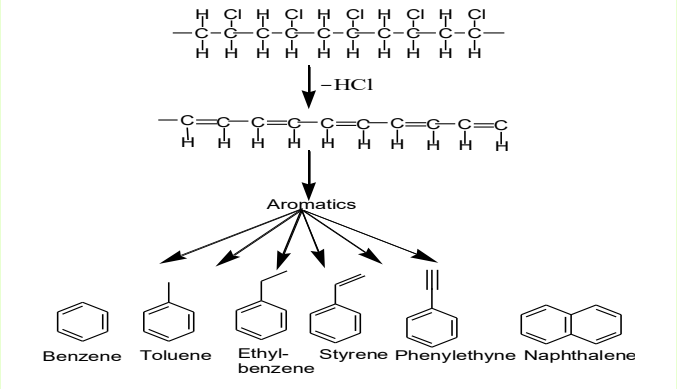


Figure 1: Side-group scission and cyclization reaction mechanism illustrated with the example of polyvinyl chloride[2]

Heating Temperature Effect

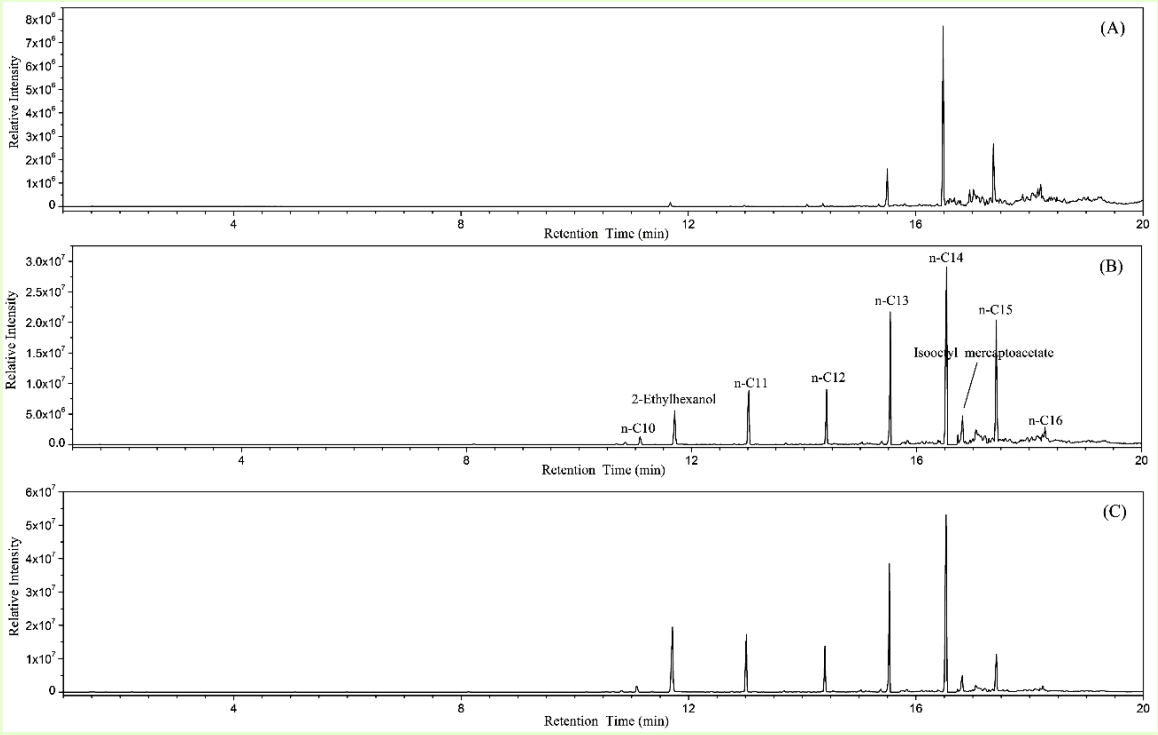


Figure 2: Standard chromatogram of PVC: (A) blank; (B) 100°C, (C) 150°C

Since PVC hardly undergoes any pyrolysis below 200°C, the substances detected at 100°C and 150°C are interferences released by PVC itself. In Figure 2, it is found that the blank analysed directly does not completely show all the volatile interferences, as some of the minor components are not detected. The headspace bottle is a relatively confined space, and has a certain concentration effect. By heating the sample at 150°C for 30 minutes in headspace bottle, the number and intensity of the volatile compounds are significantly higher than the blank.

As seen in Figure 2(B), PVC releases a large number of straight-chain alkanes, which has a certain effect on the identification of ILs such as gasoline and diesel oil. The matrix interference effects of volatile component is not as significant as pyrolysis products, but it can't be ignored.

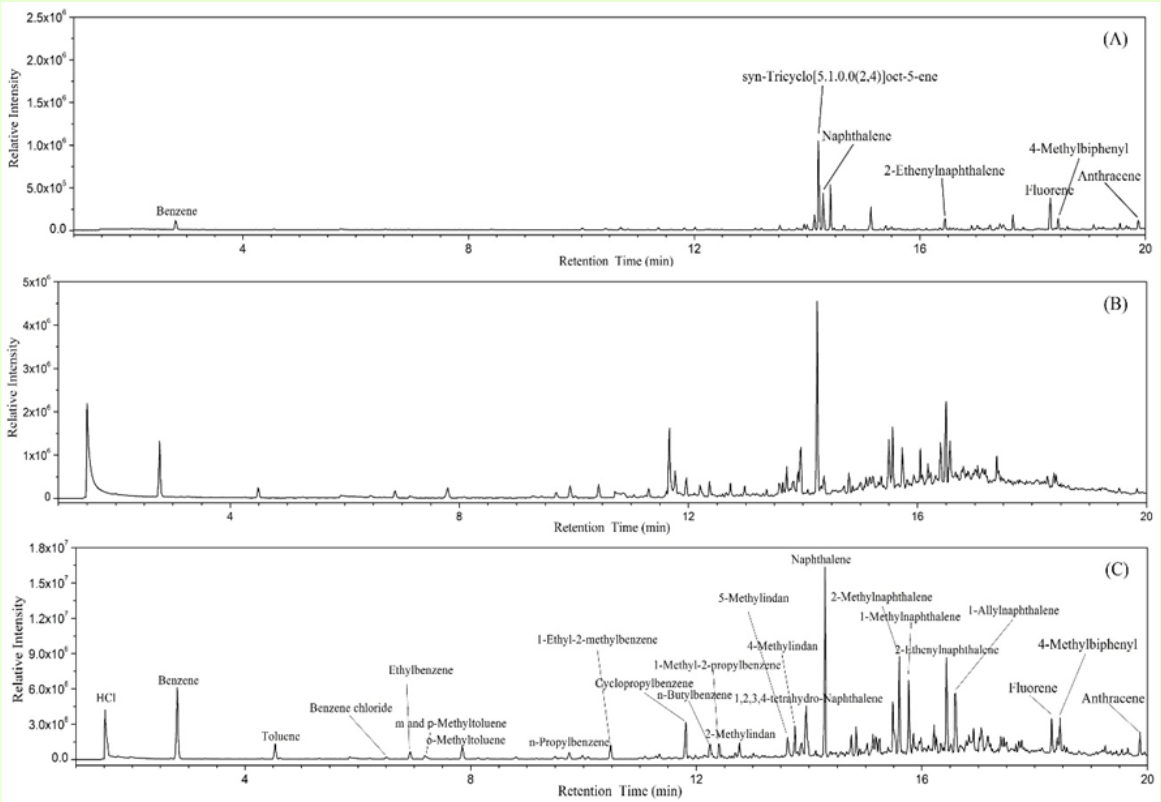


Figure 3: Standard chromatogram of PVC raw material A heated at: (A) 300° C; (B) 500°C; (C) 700°C

As seen in Figure 3(A), PVC generates small amounts of olefins and aromatic compounds at 300°C. With the gradual increase of the heating temperature, the types of pyrolysis products become more abundant, and the amount of light components increases. At 300°C, the highest peak pyrolysis product is an structurally unstable olefinic compound: syn-Tricyclo [5.1.0.0(2,4)] oct-5-ene. At 500°C and 700°C, the amount of structurally unstable olefin compounds decreased significantly, and the amount of benzene, indane, naphthalene and other compounds with relatively stable structure increased significantly.

At 700°C, PVC produces a large number of gasoline target components: benzene, toluene, xylene, mesitylene, tetramethylbenzene, naphthalene, methylnaphthalene, anthraquinone, etc, which causes a great deal of interference on the identification of gasoline debris.

Heating Time Effect

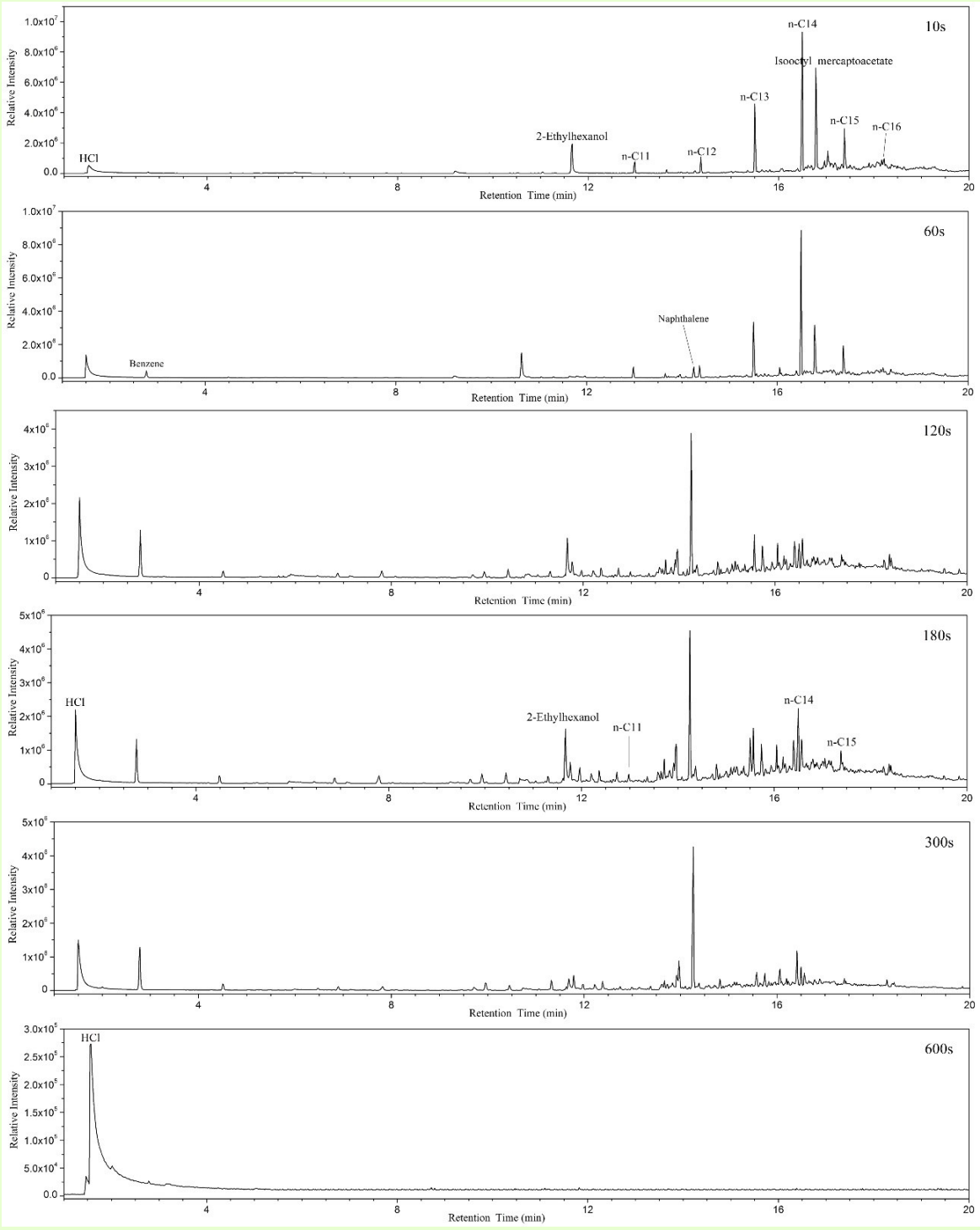


Figure 4: Standard chromatogram of PVC raw material A heated at 500°C for 10s, 60s, 120s, 180s, 300s, 600s

As seen in Figure 3(A), with the increase of heating time, the type and amount of PVC pyrolysis products initially increase and peaking at around 120 ~ 180s of heating time, before they start to decrease. At the same time, the volatile components of PVC itself gradually decrease with the increase of heating time. When we conduct heating experiments, we must pay particular attention to the fact that the pyrolysis product of the material has the highest content only during a specific heating period.

With increasing heating time, PVC pyrolysis products tend to generate structurally stable compounds-benzene, naphthalene. After heating for 60s, benzene and naphthalene can be detected, and the amount gradually increases with the heating time. Especially for naphthalene, the relative peak intensity remained the highest during the heating period from 180 to 300 seconds.

Conclusion

Through heating experiments, we improved the blank collection method and characterized the change of PVC pyrolysis products at different temperatures. Samples heated in the headspace bottle at 100°C for 30min can provide more material volatiles. The pyrolysis products are closely related to the heating temperature and time. With the gradual increase of heating temperature, the types of PVC pyrolysis products are more and more abundant. And along with the increase of heating time, the content of PVC pyrolysis products increases first and then decreases.

Acknowledgement

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Evaluation of Quantifiler Human DNA Quantification Kit using Applied Biosystems QuantStudio 5 Real-Time PCR System with HID Real-Time PCR Analysis Software v1.3.

Mr. Muhamad Sufiyan Azah, Mr. Zulhilmi Husni, Mrs. Normazlina Zainudin, Mrs. Siti Asiah Md. Nawawi, Mrs. Nor Aidora Saedon
Department of Chemistry, Malaysia

Abstract

The Quantifiler Human DNA Quantification Kit was internally validated using the Applied Biosystems QuantStudio 5 Real-Time PCR System (QS5-RTPCR) with HID Real-Time PCR Analysis Software v1.3. The study covers the standard curve and control metrics; sensitivity and stochastic; precision; accuracy; contamination; known and non-probative samples. The results demonstrate a good precision of standard curves, reproducibility of unknown samples, and sensitivity.

Introduction

The Quantifiler Human DNA Quantification Kit is among the quantification kits available for human detection purpose in DNA testing laboratories. Developmental validation of this kit was successfully performed using the Applied Biosystems 7500 Real-Time PCR Systems detection platform with Sequence Detection System (SDS) Software v1.2.3 by its manufacturer [2,3].

Internal validation of Quantifiler Human using QS5-RTPCR with HID Real-Time PCR Analysis Software v1.3 was conducted covering the standard curve and control metrics; sensitivity and stochastic; precision; accuracy; contamination; known and non-probative samples.

Materials and Methods

Standard Curve Metrics

Five plates were prepared and each plate was incorporated with two sets of standard dilution series. Each sets of standard dilution series in the plate was prepared by different analyst to see the variation of standard curve.

Sensitivity and Stochastic Study

Two sensitivity series were prepared. Sensitivity Series 1 was the genomic DNA two-fold dilution series ranging from 2 ng/µL to 3.9 pg/µL while Sensitivity Series 2 was the dilution series initiated using a neat sample with a concentration above 100 ng/µL.

Precision

Four dilution points from Sensitivity Series 1 were selected and quantified in triplicates across three different plates. The repeatability and reproducibility for concentration was assessed.

Accuracy

The concentrations obtained from neat and diluted components of National Institute of Standard and Technology (NIST) Standard Reference Material® 2372 Components A and B were compared to the reported values in the Certificate of Analysis.

Contamination

Non-template controls (NTCs) containing Tris-EDTA (TE) buffer were included in each plate to assess for contamination.

Known and non-probative samples

73 samples extracted via Chelex, Phenol-Chloroform, Automate Express (Automation) and DNA IQ™ via the Tecan Freedom Evo150 robotic platform were selected and subjected to downstream processing.

Results and Discussion

Standard Curve Metrics

All ten standard curves had slopes within the recommended range i.e. -3.3 to -2.9 and the R^2 value greater than 0.99 as well as acceptable range for y-intercept value at approximately 27.96. A relatively low standard deviation as in Table 1 for the quality metrics of all standard curves demonstrate a reproducible standard curve even though multiple operators were involved in the evaluation of the quantification process.

			Quality Metrics		
Plate	Standard Series	Columns	Slope	Y-intercept	R^2
Plate A	STD 3	1,2	-3.214	28.141	0.999
Plate A	STD 4	11,12	-3.154	28.082	0.992
Plate B	STD 3	1,2	-3.224	28.078	0.996
Plate B	STD 4	11,12	-3.144	27.964	0.996
Plate C	STD 5	1,2	-3.263	27.798	0.998
Plate C	STD 6	11,12	-3.252	27.946	0.999
Plate D	STD 1	1,2	-3.074	27.739	0.999
Plate D	STD 2	11,12	-3.227	28.165	0.998
Plate E	STD 1	1,2	-3.234	28.066	0.994
Plate E	STD 2	11,12	-2.994	27.672	0.998
		Average	-3.178	27.9651	0.9969
		Standard Deviation	0.087	0.174	0.002
		Maximum	-2.994	28.165	0.999
		Minimum	-3.263	27.672	0.992
		Fold Difference	-	1.407	-

Table 1: List of standard curve examined

Sensitivity and Stochastic Study

The Sensitivity Series 1 results are shown in Table 2 and Figure 1. Data were plotted with expected concentration on the x-axis and observed concentration on the y-axis. Higher variation was observed in the stochastic range, below the 0.023 ng/µL lowest dilution in the standard curve. The limit of detection (LOD), was observed at the concentrations of 7 pg/µL and beyond this concentration, one of the replicate was not detected.

Dilution	Expected Concentration (ng/µL)	Concentration (ng/µL)				Standard Deviation	Relative Standard Deviation
		Rep 1	Rep 2	Rep 3	Average		
A	2	1.6815	1.7066	1.6912	1.6931	0.0127	0.75%
B	1	0.96108	0.89742	0.85815	0.9056	0.0519	5.74%
C	0.5	0.38540	0.38807	0.42752	0.4003	0.0236	5.89%
D	0.25	0.22023	0.21968	0.18530	0.2084	0.0200	9.60%
E	0.125	0.08214	0.08219	0.07641	0.0802	0.0033	4.14%
F	0.0625	0.05770	0.05191	0.04203	0.0505	0.0079	15.67%
G	0.03125	0.01420	0.01499	0.01592	0.0150	0.0009	5.74%
H	0.015625	0.00848	0.01994	0.01523	0.0146	0.0058	39.56%
I	0.0078125	0.00520	0.00975	0.00502	0.0067	0.0027	40.34%
J	0.00390625	-	0.00326	0.00206	0.0027	-	-

Table 2: Concentrations for Sensitivity Series 1 dilution replicates.

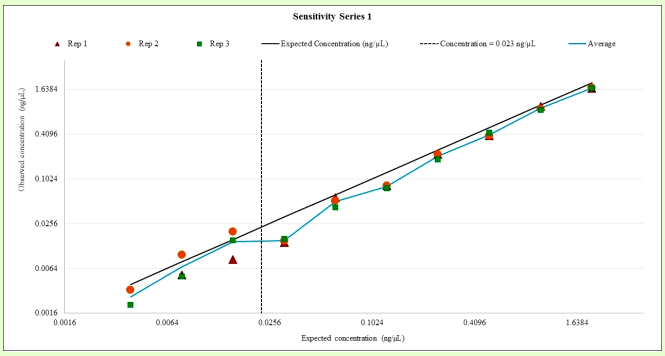

Figure 1: Concentrations for Sensitivity Series 1 dilution replicates, with a dotted line denoting the 0.023 ng/µL concentration of the lowest standard curve dilution.

The Sensitivity Series 2 results are shown in Table 3 and Figure 2. The proximity of the observed average data points to the expected average lines demonstrates the degree to which the observed results align with the expected concentrations for each dilution while the IPC values gradually increase indicating suppression of amplification as the DNA concentration rose. Neat samples show an overestimated concentration with a total suppression of IPC amplification.

Dilution	Observed Average Conc. (ng/µL)	Expected Average Conc. (ng/µL)	Percent Difference
1:32	3.9426	3.9426	-
1:16	7.4573	7.8852	-5.43%
1:8	15.261	15.770	-3.23%
1:4	30.070	31.541	-4.66%
1:2	58.087	63.082	-7.92%
Neat	160.08	126.16	26.88%

Table 3: Concentrations for Sensitivity Series 2 dilutions.

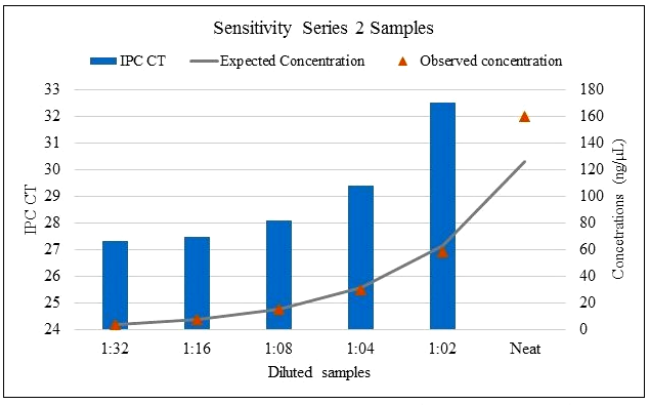

Figure 2: Average observed concentrations of the Sensitivity Series 2 dilutions

Precision

The data (in Figure 3 and Table 4) demonstrated that the observed variation among replicates quantified within a plate has the highest relative standard deviation (RSD) of 10% while the highest variation observed across three plates was 23% at all dilution points. The variation observed may be attributed to pipetting and standard curve variation between plates. This strongly suggest that the data evaluated consistent and comparable.

Dilution	Theoretical Concentration (ng/µL)	Within Plate, N=3			Across Plates, N=9		
		Average Conc. (ng/µL)	Standard Deviation	RSD	Average Conc. (ng/µL)	Standard Deviation	RSD
B	1	0.90555	0.05194	6%	0.74083	0.16065	22%
C	0.5	0.40033	0.02358	6%	0.33210	0.06904	21%
D	0.25	0.20840	0.02001	10%	0.16622	0.03835	23%
E	0.125	0.08025	0.00333	4%	0.07284	0.01227	17%

Table 4: Precision for concentration within a plate and across three plates.

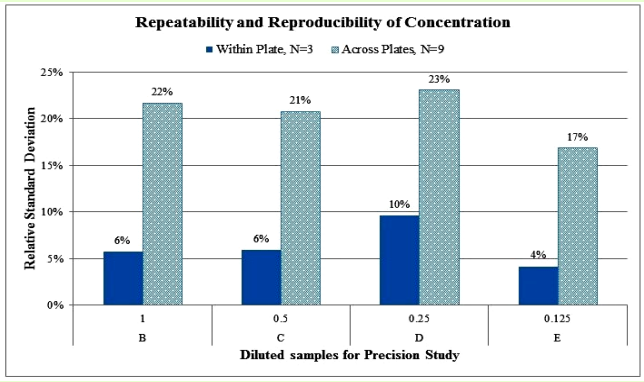

Figure 3: RSD of diluted samples within and across plates.

Accuracy

The observed concentration for the diluted 2372 Component A is 10% lower than the expected concentration with the RSD at 4%, whereas Component B is 14% higher with RSD at 12% (Table 5). Therefore, it shows that quantification using Quantifiler Human kit via QS5 is accurate as the concentration determined does not underestimate or overestimate beyond 50% [6].

Contamination

A total of 28 NTCs were quantified with only one positive signal detected and reported concentration of 125.23 ng/µL. Review on the multicomponent plot between NTC-TE and the highest standard concentration demonstrate an abnormal curve (Figure 4). In a normal multicomponent curve, FAM™ fluorescence signal will shows exponential rise as the template DNA is amplified [7]. However, FAM™ fluorescence signal in this NTC did not exponentially increase and instead plateau after crossing threshold limit which indicate no amplification. Thus positive signal in this NTC may have derived from some unknown source instead of template DNA.

Known and non-probative samples

None of the samples showed undetermined human target concentrations. Two samples indicated possible inhibition, having an IPC Cᴛ value that was undetermined. These undetermined IPC Cᴛ samples developed in-scale profiles which indicate a reliable result.

Conclusion

The Quantifiler Human through QuantStudio 5 Systems provides quantitative results for a variety of sample types with good precision of standard curves, reproducibility of unknown samples, and sensitivity. The assay also provides sample quality information, with indicators for potential PCR inhibition. All this information is useful for downstream analysis.

Sample	Average Concentration (ng/µL)	Standard Deviation (ng/µL)	RSD	Reference Concentration (ng/µL)	Percent Difference
2372 A 1:10	6.2913	0.26621	4%	5.7	10%
2372 A NEAT	83.426	15.9497	19%	57	46%
2372 B 1:10	5.2586	0.62482	12%	6.1	-14%
2372 B NEAT	75.912	2.9798	4%	61	24%

Table 5: Average concentration of NIST components and percent differences.

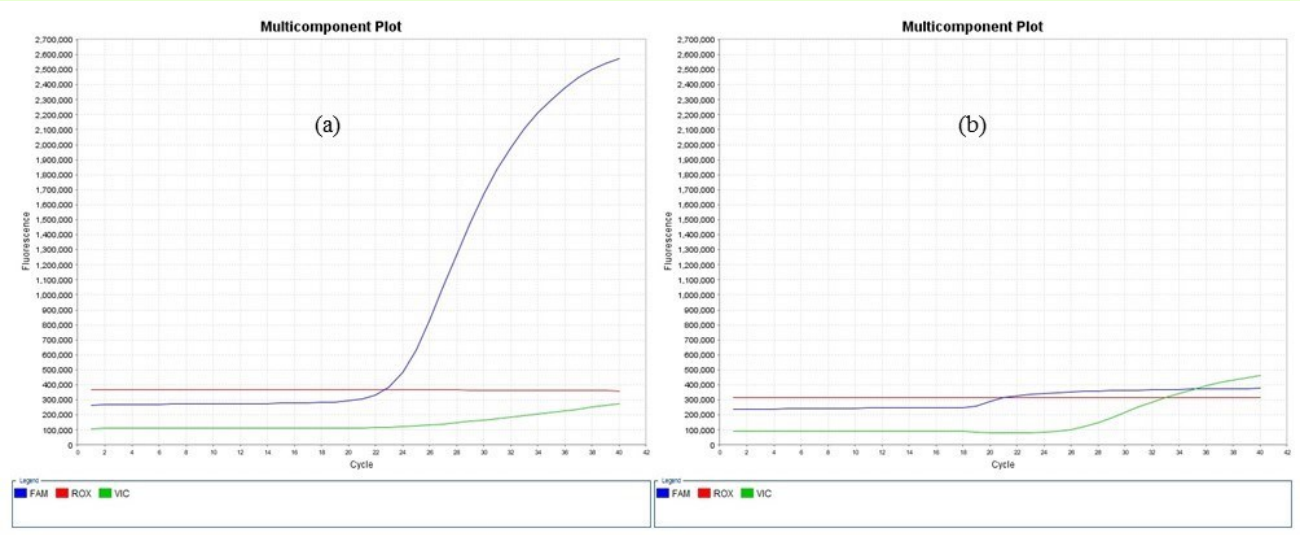

Figure 4: Comparison between multi-component plot from (a) 50ng/µL standard series and (b) the positive signal NTC.

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Genetic Polymorphism of 24 STR Loci for the Chinese Population in Macao

Fei SA, Tek LAO, Chon Lan WONG
Forensic Science Department of Judiciary Police, Macau SAR, People's Republic of China

Abstract

Allele frequencies for 24 STR loci, namely D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338, Penta E, Penta D and D6S1043, which were tested by Applied Biosystem's GlobalFiler® Express PCR Amplification Kit, AmpFlSTR® Sinofiler™ PCR Amplification Kit and Promega's PowerPlex® 16 System, were obtained from 219 unrelated local Chinese in Macao. No deviations from Hardy-Weinberg equilibrium were observed for all loci. The matching probability and the combined power of exclusion for the 24 tested STR loci were $1\text{-}1.7688\times10^{-29}$ and 0.999 999 999 989 104, respectively.

Population: Buccal samples of 219 unrelated volunteers from local Chinese population in Macao were collected with informed consent.

DNA extraction: Genomic DNA was extracted from buccal samples using the Chelex-100 method.

PCR amplification: Commercial DNA typing kits, GlobalFiler® Express PCR Amplification Kit (Applied Biosystems), AmpFlSTR® Sinofiler™ PCR Amplification Kit (Applied Biosystems), and PowerPlex® 16 System (Promega) were used. PCR amplifications were carried out on GeneAmp® PCR system 9700 (Applied Biosystems).

Cycling conditions: GlobalFiler® Express PCR Amplification Kit was used according to the User's Manual and 26 cycles was adopted. AmpFlSTR® Sinofiler™ PCR Amplification Kit was used according to the User's Manual. PowerPlex® 16 System was used according to the User's Manual and 30 cycles was adopted.

Typing: Amplification products were detected in a ABI PRISM® 3100 Genetic Analyzer or AB 3130xL Genetic Analyzer or AB 3500xL Genetic Analyzer (Applied Biosystems). Allele calling was performed using GeneMapper ID v3.2 or GeneMapper ID-X v1.4 (Applied Biosystems).

Quality control: Laboratory internal control standards and kit control were employed.

Analysis of data: Allelic frequencies, the matching probability (MP), the power of discrimination (PD), the probability of paternity exclusion (PE), the polymorphism information content (PIC), Typical Paternity Index (TPI), and the heterozygosity (H) were calculated using the PowerStats v1.2 [1]. The exact test for Hardy–Weinberg Equilibrium (HWE) and the exact test for pair-wise linkage equilibrium analysis were conducted using Power Marker software v3.25 [2].

Results: Allelic frequencies are shown in Table 1, MP, PD, PE, PIC, TPI, H, and p-values from exact test for HWE are shown in Table 2. There was no deviation from Hardy-Weinberg Equilibrium observed for D6S1043 as previously reported [3]. For the pair-wise linkage equilibrium analysis, the exact test detected 13 departures from independence out of 276 comparisons, but all p-values were not significant after Bonferroni's correction. The matching probability of the 24 loci is $1\text{-}1.7688\times10^{-29}$. The combined PE of the 24 loci is 0.999 999 999 989 104.

Other remarks: The genotype result of one test sample at D5S818 was 10 using AmpFlSTR® Sinofiler™ PCR Amplification Kit but was 10,11 using PowerPlex® 16 System and GlobalFiler® Express PCR Amplification Kit. This non-concordance may due to the difference in primer sequence in these three kits. This sample was excluded in the test results.

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SE33		Penta E		FGA		D6S1043		D18S51		D21S11		D12S391	
A	F	A	F	A	F	A	F	A	F	A	F	A	F
14	0.002	5	0.053	13	0.007	9	0.002	11	0.002	27	0.009	15	0.007
14.2	0.002	7	0.002	16	0.007	10	0.027	12	0.030	28	0.053	16	0.007
15	0.007	8	0.002	18	0.014	11	0.139	13	0.164	29	0.260	17	0.064
16	0.034	9	0.011	19	0.094	12	0.144	14	0.205	29.2	0.002	18	0.231
17	0.030	10	0.046	20	0.050	12.3	0.002	15	0.174	30	0.249	19	0.231
18	0.082	11	0.212	21	0.142	13	0.144	16	0.178	30.2	0.011	20	0.194
19	0.100	12	0.098	21.2	0.005	14	0.151	17	0.091	31	0.098	21	0.078
20	0.046	13	0.055	22	0.169	15	0.021	18	0.041	31.2	0.103	22	0.080
20.2	0.005	14	0.062	22.2	0.005	16	0.002	19	0.046	32	0.023	23	0.068
21	0.043	15	0.096	23	0.171	17	0.027	20	0.021	32.2	0.146	24	0.027
21.2	0.016	16	0.062	23.2	0.009	18	0.139	21	0.021	33.2	0.039	25	0.011
22	0.007	17	0.071	24	0.158	18.2	0.005	22	0.011	34.2	0.007	27	0.002
22.2	0.030	18	0.064	24.2	0.011	18.3	0.002	23	0.014	D2S441		D10S1248	
23	0.005	19	0.048	25	0.075	19	0.148	25	0.002	A	F	A	F
23.2	0.064	19.4	0.007	25.2	0.009	20	0.034	D13S317		9.1	0.014	8	0.002
24	0.002	20	0.048	26	0.053	21	0.011	A	F	10	0.228	10	0.005
24.2	0.080	21	0.027	26.2	0.002	D1S1656		6	0.002	10.1	0.007	11	0.002
25.2	0.059	22	0.016	27	0.018	A	F	7	0.002	11	0.324	12	0.050
26.2	0.064	23	0.005	28	0.002	11	0.091	8	0.295	11.1	0.002	13	0.354
27.2	0.064	24	0.009	D19S433		12	0.039	9	0.142	11.3	0.075	14	0.224
27.3	0.002	25	0.005	A	F	13	0.091	10	0.164	12	0.183	15	0.244
28.2	0.084	28	0.002	9	0.005	14	0.091	11	0.221	13	0.030	16	0.100
29.2	0.062	vWA		11	0.009	15	0.276	12	0.128	14	0.126	17	0.016
30.2	0.046	A	F	12	0.046	15.3	0.005	13	0.039	15	0.009	18	0.002
31.2	0.039	13	0.002	12.2	0.007	16	0.203	14	0.007	16	0.002	D7S820	
32.2	0.014	14	0.292	13	0.276	16.3	0.009	Penta D		D8S1179		A	F
33.2	0.009	15	0.025	13.2	0.034	17	0.066	A	F	A	F	7	0.009
37.2	0.002	16	0.153	14	0.288	17.3	0.087	7	0.014	10	0.114	8	0.162
D3S1358		17	0.224	14.2	0.105	18	0.018	8	0.073	11	0.167	9	0.055
A	F	18	0.208	15	0.037	18.3	0.018	9	0.358	12	0.128	9.1	0.002
13	0.005	19	0.078	15.2	0.148	19.3	0.005	10	0.132	13	0.155	9.2	0.002
14	0.041	20	0.016	16	0.005	D2S1338		11	0.128	14	0.164	10	0.148
15	0.288	21	0.002	16.2	0.039	A	F	12	0.137	15	0.178	11	0.370
16	0.336	D22S1045		17.2	0.002	16	0.018	13	0.110	16	0.082	12	0.210
17	0.276	A	F	CSF1PO		17	0.066	14	0.041	17	0.011	13	0.041
18	0.050	11	0.146	A	F	18	0.098	15	0.007	D5S818		D16S539	
19	0.005	12	0.005	7	0.007	19	0.192	TH01		A	F	A	F
TPOX		13	0.002	9	0.048	20	0.135	A	F	7	0.032	8	0.007
A	F	14	0.046	10	0.226	21	0.046	6	0.107	9	0.073	9	0.242
8	0.584	15	0.352	11	0.276	22	0.059	7	0.290	10	0.196	10	0.151
9	0.082	16	0.212	12	0.345	23	0.185	8	0.053	11	0.326	11	0.260
10	0.021	17	0.205	13	0.080	24	0.135	9	0.470	12	0.212	12	0.224
11	0.301	18	0.030	14	0.016	25	0.057	9.3	0.039	13	0.148	13	0.105
12	0.009	19	0.002	15	0.002	26	0.009	10	0.041	14	0.011	14	0.011
13	0.002												

Table 1: Allele frequencies of 24 STR loci for Chinese population in Macao (n = 219)

	MP	PD	PE	PIC	TPI	H	p-value
D3S1358	0.131	0.869	0.516	0.673	2.028	0.753	0.534
vWA	0.079	0.921	0.614	0.760	2.607	0.808	0.677
D16S539	0.078	0.922	0.572	0.757	2.330	0.785	0.981
CSF1PO	0.116	0.884	0.581	0.702	2.380	0.790	0.328
TPOX	0.254	0.746	0.224	0.492	1.084	0.539	0.373
D8S1179	0.043	0.957	0.685	0.835	3.221	0.845	0.395
D21S11	0.057	0.943	0.649	0.802	2.882	0.826	0.549
D18S51	0.042	0.958	0.721	0.838	3.650	0.863	0.430
D2S441	0.078	0.922	0.524	0.756	2.066	0.758	0.103
D19S433	0.082	0.918	0.712	0.776	3.532	0.858	0.813
TH01	0.156	0.844	0.392	0.629	1.542	0.676	0.461
FGA	0.033	0.967	0.767	0.864	4.380	0.886	0.140
D22S1045	0.096	0.904	0.572	0.729	2.330	0.785	0.526
D5S818	0.081	0.919	0.556	0.749	2.235	0.776	0.470
D13S317	0.071	0.929	0.614	0.770	2.607	0.808	0.966
D7S820	0.088	0.912	0.540	0.733	2.147	0.767	0.504
SE33	0.012	0.988	0.870	0.935	7.821	0.936	0.126
D10S1248	0.099	0.901	0.500	0.713	1.955	0.744	0.890
D1S1656	0.044	0.956	0.712	0.826	3.532	0.858	0.478
D12S391	0.055	0.945	0.739	0.813	3.911	0.872	0.172
D2S1338	0.034	0.966	0.694	0.856	3.318	0.849	0.416
Penta E	0.020	0.980	0.794	0.899	4.977	0.900	0.085
Penta D	0.066	0.934	0.640	0.777	2.808	0.822	0.765
D6S1043	0.035	0.965	0.813	0.858	5.475	0.909	0.631

Table 2: Statistical parameters of 24 STR loci for Chinese population in Macao (n = 219)

Inferring cell-origin from sperm and non-sperm DNA extracts: A fallacy

Dr. Seah Lay Hong, Mr. Phoon Yoong Keat, Mr. Wee Boon Hui
Department of Chemistry, Malaysia

Abstract

Differential extraction of semen stains is intended to separate the epithelial DNA from the sperm DNA into the non-sperm and sperm extracts respectively. However, the differential profiles of stains containing entirely of sperm mixtures (as usually encountered in gang-rape) revealed sperm DNA even in the non-sperm extracts. Experiments with controlled amounts of sperm mixtures on cloth and FTA cards demonstrated the DNA of the major sperm contributor persisted in the sperm extract and the DNA of the minor sperm contributor(s) is usually detected only in the non-sperm extract. The observation that classical differential extraction of sperm DNA mixtures can extract sperm DNA without the addition of disulfide cleaving agent like dithiothreitol debunk the current concept of stabilization of sperm chromatin by disulfide bridges which forms the basis of separation of non-sperm and sperm DNA by differential extraction. Consequently, there is a need to review the terms of reference for the two fractions using the classical differential extraction technique.

Introduction

The differential extraction procedure first described by Gill et al. [1] refers to the process by which the DNA from epithelial cells and sperm cells can be separated into different fractions.

In the investigation of cases of sexual assault, semen stains on vaginal swabs, clothing and bedding items provide the most incriminating evidence. In a gang-rape scenario, the elucidation of the perpetrators becomes more complex due to the presence of multiple contributors in the semen stains. [2] Semen stains on bedding or clothing items could invariably consist of sperm DNA from multiple sources. When differential extraction is performed on these stains, the DNA in both epithelial and sperm fractions would logically be of sperm origin.

In this study, semen from different sources was separately dried on cotton cloth and FTA cards. Differential extraction was carried out on major-minor mixtures of sperm. The differential profiles were analyzed to determine the separation patterns.

Materials and Method

The differential extraction protocol was carried in extraction buffer (10 mM Tris-HCl, 10 mM EDTA and 10 mM NaCl, pH 8) with 2 % SDS using a one-tenth volume of 10 mg/ml Proteinase K for the non-sperm extract and a one-tenth volume of 0.39 M DTT (dithiothreitol) for the sperm extract. Digestion and incubation was carried out at 37^{o}C for 1-2 hr for the non -sperm extract and 3 hr for the sperm extract.

Semen from three voluntary donors were stained separately on white polyester-viscose cloth and FTA cards. Organic extraction was carried out on 1.2-mm and 2-mm disc punches of the semen-stained cloth and FTA cards. The DNA was then quantified using the ABI Quantifiler® Human DNA Quantification Kit.

Six different sperm mixtures were then set up comprising of semen on cloth as well as FTA cards. Each mixture is represented by a major and minor(s) contributors where DNA estimates were from quantification of discs punched from the semen-stained cloth and FTA cards. Differential extraction was carried out on the six mixtures. Template DNA (1 ng estimate) was amplified with AmpFlSTR®Identifiler® Plus amplification kit for 28 PCR cycles according to manufacturer's instructions. The amplicons were separated on an Applied Biosystems 3130xl Genetic Analyzer with CE injection at 3 kV/10 s and analyzed using GeneMapper ID v3.2.1 software. A detection threshold limit of 50 rfu was used to designate alleles.

Results and Discussion

In the first four two-source sperm mixtures on cloth comprising of one major and one minor contributor (see Table 1) with major-minor ratio ranging from 6:1 to 13:1, the differential DNA extraction results revealed the major contributor to persist in the sperm fraction. The DNA profile of the minor contributor was detected and could only be read in the non-sperm fraction. In the three-source sperm mixtures on FTA cards (SSM1_5 and SSM1_6 in Table 1), similar separation patterns was observed. The major contributor persists in the sperm fraction; the minor peaks in the sperm fraction are not reportable. The other two minor contributors could only be read in the non-sperm fraction.

This is an interesting phenomenon. Of important significance is the observation that sperm DNA can be successfully extracted into the non-sperm extract without the addition of a disulfide cleaving agent like dithiothreitol. There appears to be preferential extraction of the minor sperm contributor in the non-sperm extract [3]. A strict interpretation would infer the DNA of the minor sperm contributor in the non-sperm extract to be from non-sperm cell origin. The observed disruptive change in differential extraction behavior for sperm DNA cannot be adequately explained by the currently accepted disulfide bridge-dependent model. [4]

This current concept on sperm chromatin stability has been challenged by Björndahl and Kvist [5,6] who proposed an alternative model of a zinc dependent chromatin stability with formation of zinc bridges between protamine thiols and potentially imidazole groups of histidine. The zinc-dependent chromatin stability is rapidly lost during the DNA extraction process for the epithelial fraction where the use of surfactants like SDS (sodium dodecyl sulphate) and EDTA in the extraction buffer provides an effective zinc chelating medium. The loss of zinc causes decondensation of the sperm chromatin [7] and subsequently, rupture of the sperm heads and release of sperm DNA into the non-sperm or epithelial extract. As observed in this study for major-minor sperm mixtures (Table 1), the minor sperm population is seen to have a weaker zinc-dependent chromatin stability compared to the more resilient major sperm population which arguably possess a relatively tighter packing of the sperm DNA chromatin fibers.

Conclusion

Interpretation of sperm mixtures commonly encountered in gang-rape thus requires a careful consideration of the separation profiles derived from differential extraction. The detection of the minor sperm contributor in the non-sperm extract impacts on inferring cell-origin of DNA in the non-sperm extract. This fallacy requires an assessment of the terms of reference for the two fractions: non-sperm and sperm extracts.

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Marking	Semen Mixture	Non-sperm Extract	Sperm Extract
SSM1_1	[M1(a)]: [M2(a)] 12 : 1	[M1(a)]+ [M2(a)] [M1(a)] = major	[M1(a)] dominant [M2(a)] not reportable
SSM1_2	[M1(a) : [M2(a)] 6 : 1	[M1(a)]+ [M2(a)] [M1(a)] = minor	[M1(a)] dominant [M2(a)] not reportable
SSM1_3	[M1(a)] : [M3(a)] 13 : 1	[M1(a)]+ [M3(a)] [M1(a)] = minor	[M1(a)] dominant [M3(a)] not reportable
SSM1_4	[M1(a)] : [M3(a)] 7 : 1	[M1(a)]+ [M3(a)] [M1(a)] = minor	[M1(a)] dominant [M3(a)] not reportable
SSM1_5	[M1] : [M2(b)] : [M3] 13 : 6.3 : 1	[M1] +[M2(b)] +[M3]	[M1] major [M2(b)] minor [M3] not reportable
SSM1_6	[M1] : [M2(b)] : [M3] 11 : 2.3 : 1	[M1] +[M2(b)] +[M3] [M3] = Major	[M1] major [M2(b)] not reportable [M3] not reportable

Table 1: Results from differential extraction of semen mixtures. Three donors were depicted by M1 or M1(a), M2(a) or M2(b), M3 or M3(a). The abbreviations (a) or (b) were used to indicate different stained materials.

Research of the reaction mechanism of heat induced fluorescence imaging for fingerprint sweat components

Mr. Li Xiaojun, Ms .Xue Jing, Dr. Ma Rongliang, Ms. Qu Huiying, Mr. Liu Huan
Institute Of Forensic Science, People's Republic of China

Abstract

Heat induced fluorescence imaging technology is a non-destructive and non-toxic identifying method of latent sweat fingerprint on paper. It is chemical reagent free, quick and easy, and can be applied in conjunction with a wide variety of traditional enhancement methods. Through exploring the effects of different parts of sweat to the effect of fluorescence, the reaction mechanism of the heat induced fluorescence imaging technology can be analyzed and demonstrated. By using paper, foil, glass slides and metal surface as a carrier, we examined whether the paper, sodium chloride, protein, glucose and amino acids, for example, would affect forming of the fluorescence phenomenon. The heat induced fluorescence reaction of three kinds of amino acids was first introduced in theory on the basis of determining the fluorescence reaction, and the correctness of the conclusion is verified by GC-MS. Shown by the experimental results, the fluorescent substance is mainly composed of a variety of amino acids in sweat. A further finding is that glycine, alanine, and phenylalanine can generate a rigid six membered ring of the fluorescent substance by dehydration polymerization under the condition of heating. This is to promote the development of this technology in the field of fingerprint and plays an important role.

Introduction

Heat induced fluorescence imaging technology can well show the latent sweat fingerprints on various kinds of paper, and its fluorescent effect is better than DFO and indanedione. The technology that is quick and easy can show latent sweat fingerprint on complex and dark background paper. That is, after a short heating at the right temperature, placing under the excitation environment of blue and green light (wavelength of 415-510nm), and through the observation of orange goggles, a strong yellow fluorescent fingerprint is observed. Heat induced fluorescence imaging technology, without chemical reagents, does not impact paper samples, and is not harmful to technical staff members. And the technology will not affect the performance of traditional chemical methods. With these advantages, the technology will become an important pretreatment method that shows the latent sweat fingerprints on paper[1-3]. However, there are very few studies on the reaction mechanism of the heat induced fluorescence imaging latent sweat fingerprint on paper at home and abroad, and no complete set of reaction mechanisms as the theoretical support of this technology. Therefore, its reaction mechanism is worth further research and exploration, in order to theoretically explain clearly the origin of this phenomenon. The combination of theory and practice will be more favorable for the popularization and use of the heat induced fluorescence imaging technology.

Materials and Methods

Major instruments and consumables: BRUKER-VERTEX-70 type Fourier transform infrared spectrometer, Gas chromatography - mass spectrometry 2010Plus (Shimadzu Corporation), III 500M AVANCE liquid nuclear magnetic resonance spectrometer (Brook company), Heat induced fluorescence heating device, Multi band observation camera equipment, Canon SLR camera, Orange filter, pipette, electronic balance, foil, glass slides, white paper, black cardboard.

Reagents

Artificial sweat (In accordance with the relevant literature), Sodium chloride, distilled water, 11 kinds of amino acid standards (Glycine, alanine, serine, threonine, leucine, isoleucine, phenylalanine, proline, valine, tryptophan, tyrosine), Methanol (Chromatographic), 2,5-piperazinedione (Purity greater than 99%), 3,6-dimethylpiperazine-2,5-dione (Purity greater than 97%), Cyclo (L-Phe-Phe) (purity greater than 99%).

GC-MS conditions

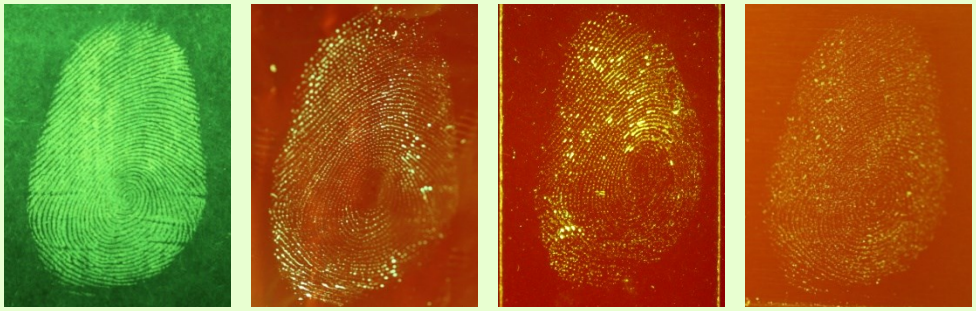
DB-5ms (0.25um * 0.25mm * 30m); The carrier gas is helium, the purity is 99.995%, the flow rate is 1.00mL/min. The initial column temperature is 80°C, the retention of 2min, to 30°C/min temperature programmed to 280°C, reserve 17min, ion source type EI, source temperature 200°C, interface temperature 200°C, the quality of the scan range: 40-450m/z.

Results and Discussion

The impact of bearing mark objects

As shown in figure 1, sweat fingerprints on different bearing mark objects can be observed better with fluorescence after proper heat treatment. The fact that fluorescence is observed on foil, glass slides and metal surface as the bearing mark objects eliminates paper as a contributor to the reaction. Experimental results show 4 pieces of sweat fingerprints on these three kinds of bearing mark objects in addition to paper

and the fluorescence intensity for foils, glass slides and metal surfaces is not less than the white copy paper's. In the absence of paper, sweat fingerprint can also demonstrate heat induced fluorescence. The fluorescent substance is generated by some ingredients in sweat.



white paper *foil* *glass slides* *metal surface*

Figure 1. Heat induced fluorescence effect of sweat fingerprints on several different objects.

The effect of sodium chloride

According to relevant literature reports, sodium chloride as one of the main ingredients in the sweat may also be involved in the fluorescence reaction[4]. According to this conclusion, the experiment is verified by comparative analysis. The results are shown in figure 2, three groups of samples with sodium chloride and without sodium chloride artificial sweat all produced a fluorescent effect of similar intensity. Therefore, sodium chloride has no observable effect on the fluorescence of sweat and does not participate in the reaction.

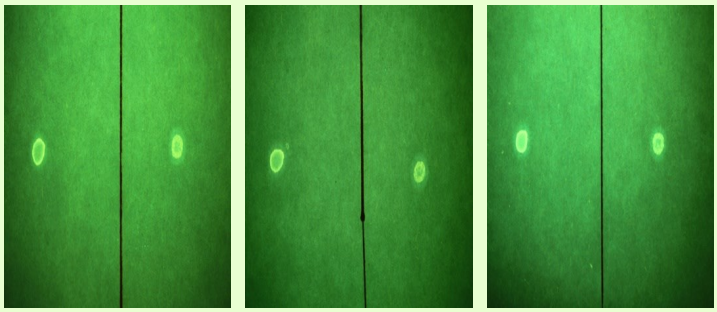


Figure 2: Comparison of Sodium chloride influence sweat heat induced fluorescence effect (the left side is not added with sodium chloride, and the right is added with sodium chloride)

The effects of protein and glucose

Protein and glucose as parts of the sweat are eliminated as contributing to the fluorescent effect. According to the characteristics of fluorescent substance, protein and glucose do not possess structural characteristics necessary for the formation of fluorescent substances. In the experiment, the fluorescence of protein and glucose was not observed, which verifies the fact that these two substances are not involved in the heat induced fluorescence reaction.

The effects of amino acids

Sweat contains rich amino acids. In the "Chinese Criminal Science and technology: fingerprint technology," a book, it is mentioned that there are 11 kinds of amino acids in sweat, such as glycine, alanine, serine, threonine, leucine, isoleucine, phenylalanine, proline, valine, lubricious ammonia acid, tyrosine. And the concentrations of most amino acids in human sweat are in the range 0.01 ~ 0.04 mg/ml[5]. The following experiments were carried out to verify the results of these 11 amino acids. As shown in figure 3, after heated treatment, 11 kinds of amino acids all demonstrate the fluorescence effect. Compared to the heating induced fluorescence effect of various kinds of amino acids, the fluorescence effect of leucine, isoleucine, and tyrosine is poorer, but it is still observable. From fluorescence effect of different concentrations of amino acid, the amino acid fluorescence effect of 2mg/ml was better than 0.2mg/ml and 0.2mg/ml was better than 0.02mg/ml. The presence of a single amino acid can also generate fluorescent substances, and the fluorescence effect is directly proportional to the concentration of amino acid.

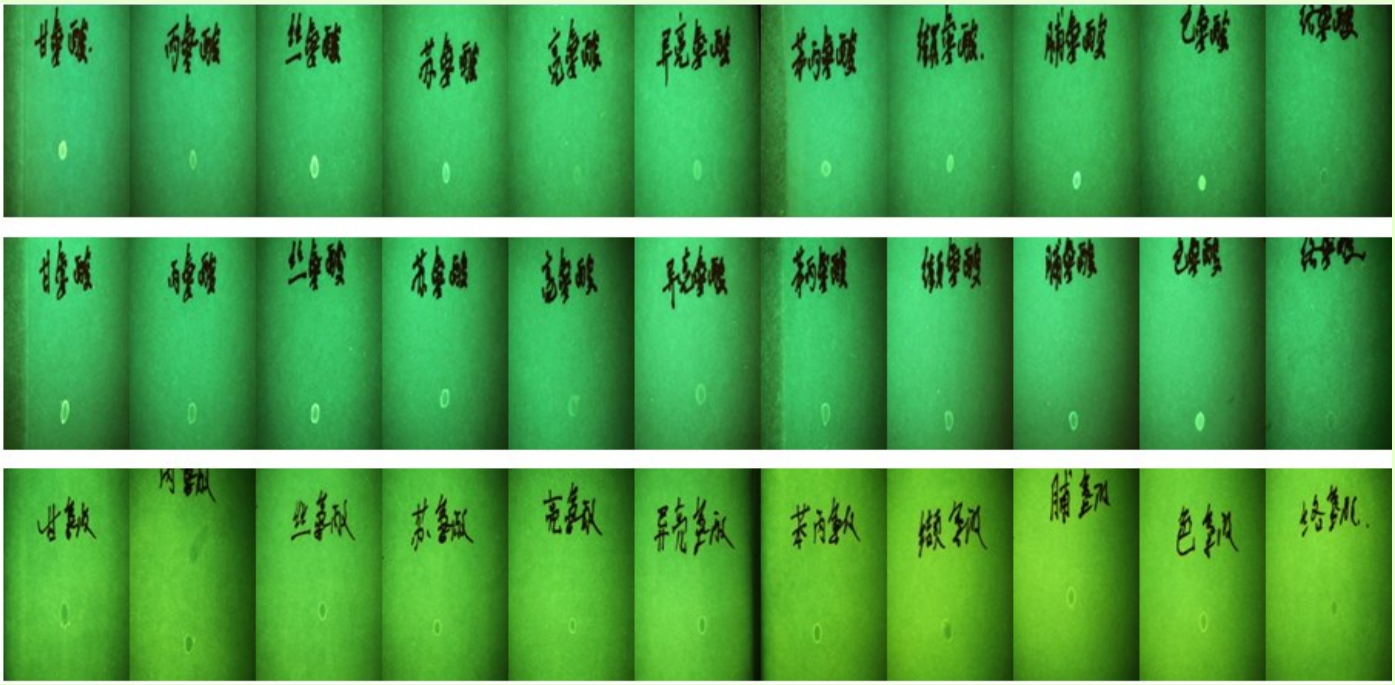


Figure 3: Heat induced fluorescence effect of different concentrations of amino acids on the white paper (Upper: 2mg/ml, medium: 0.2mg/ml, down: 0.02mg/ml)

GC-MS analysis

Figures 4, 6 and 9, respectively, are GC-MS total ion flow diagrams of 2,5-piperazinedione、3,6-dimethylpiperazine-2,5-dione and Cyclo (L-Phe-Phe), and their retention time was 7.301, 6.965 and 13.659. The fragment mass spectra of three kinds of standard products were 43, 71, 86 and 114; 44, 99, 142 and 91; and 120, 175, 203 and 294 respectively. Figure 7 and 10 are GC-MS total ion flow diagrams and corresponding amino acid mass spectra of alanine and phenylalanine. Figure 5, 8 and 11 left are GC-MS total ion flow diagrams of 2,5-piperazinedione、3,6-dimethylpiperazine-2,5-dione and Cyclo (L-Phe-Phe) that are produced by appropriate heating of glycine, alanine, and phenylalanine. It can be seen that the heating product of the three kinds of amino acids is a mixture of many kinds of substances including amino acids and amino acid heating product. The same retention time in Figure 5, 8 and 11 demonstrated the target peaks and new peaks compared with the spectrum of amino acids. It shows that the substance is a new substance produced by heating the amino acids whose mass spectra are shown in the right side of Figure 5, 8 and 11 and the fragment mass spectra of which were also 43, 71, 86 and 114; 44, 99 and 142; and 91, 120, 175, 203 and 294 respectively. They are consistent with the fragment mass spectra of three kinds of standard products. From the above analysis, we can see that the two substances include the same derivative, and this derivative is the fluorescent substance derived from theory. In addition, it can also be seen that the content of fluorescent substances in the mixture is less through the size of the peak area. Therefore, the results of GC-MS analysis confirmed that the fluorescence reaction processes of glycine, alanine and phenylalanine that are derived theoretically are completely correct.

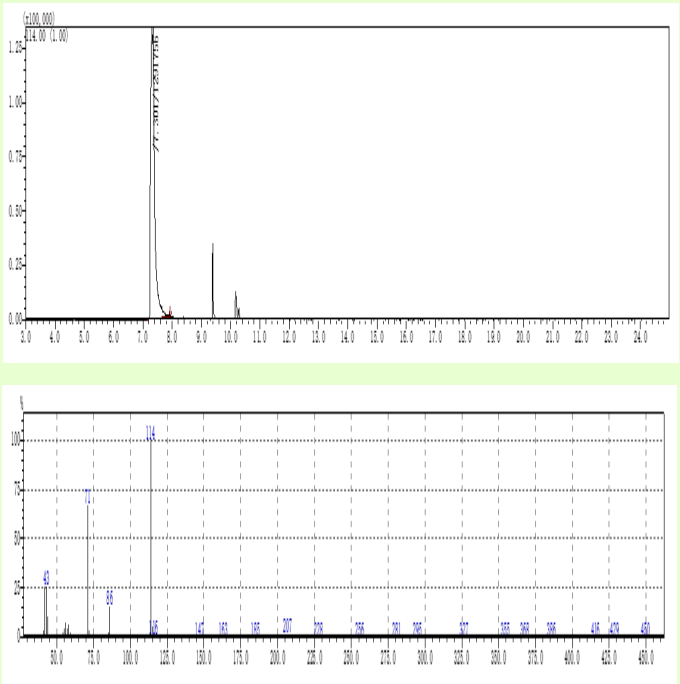


Figure 4: GC-MS total ion flow diagram (left) and mass spectra (right) of 2,5-piperazinedione.

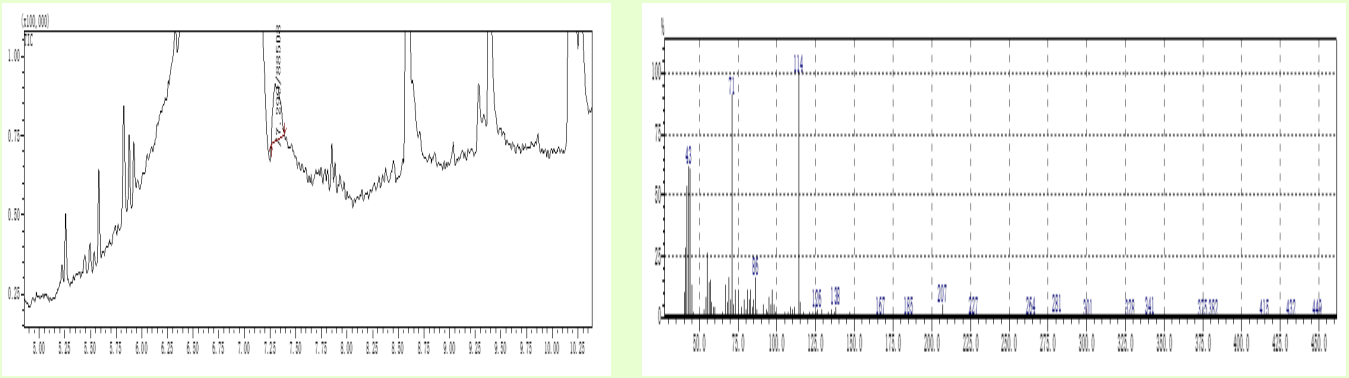


Figure 5: GC-MS total ion flow diagram (left) and mass spectra (right) of 2,5-piperazinedione by heating glycine.

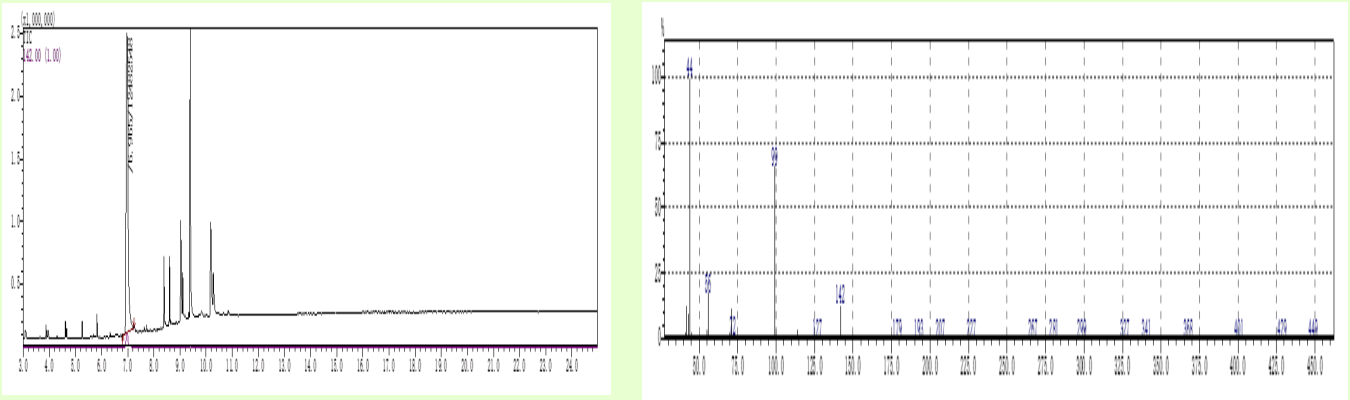


Figure 6: GC-MS total ion flow diagram (left) and mass spectra (right) of 3,6-dimethylpiperazine-2,5-dione.

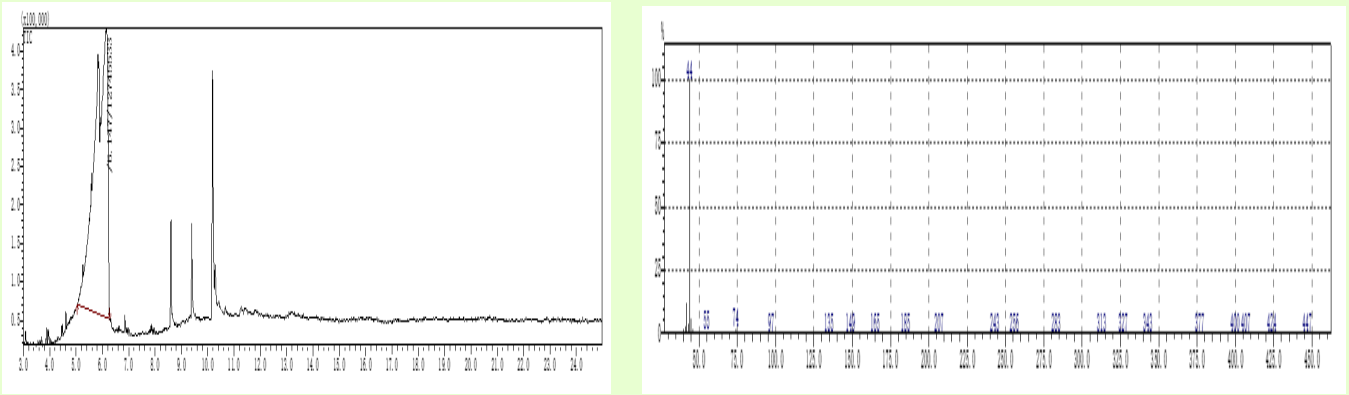


Figure 7: GC-MS total ion flow diagram (left) and mass spectra (right) of alanine.

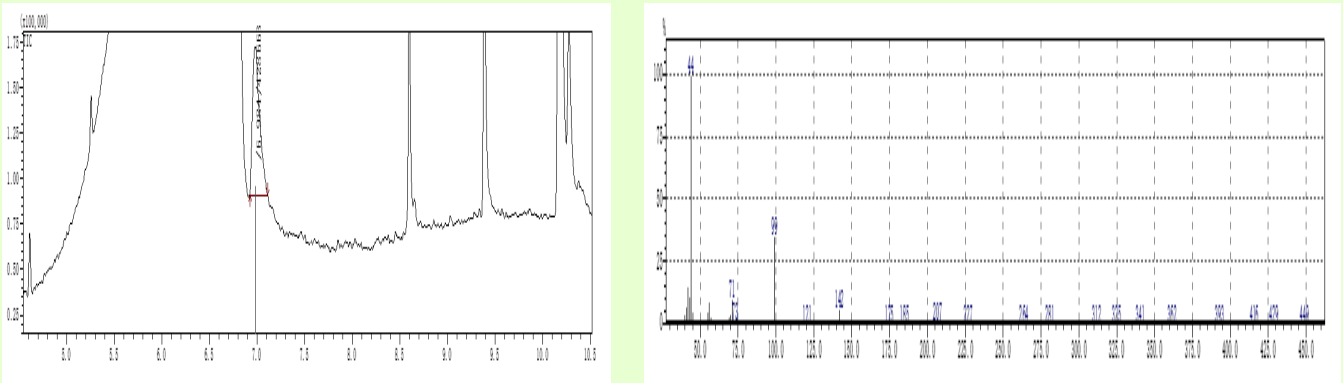


Figure 8: GC-MS total ion flow diagram (left) and mass spectra (right) of 3,6-dimethylpiperazine-2,5-dione by heating alanine.

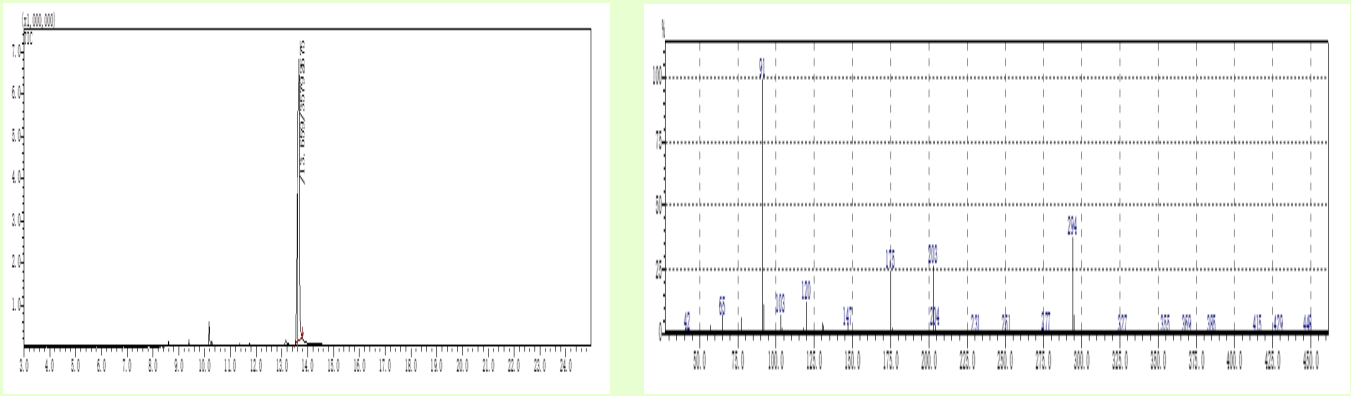


Figure 9: GC-MS total ion flow diagram (left) and mass spectra (right) of Cyclo(L-Phe-Phe.

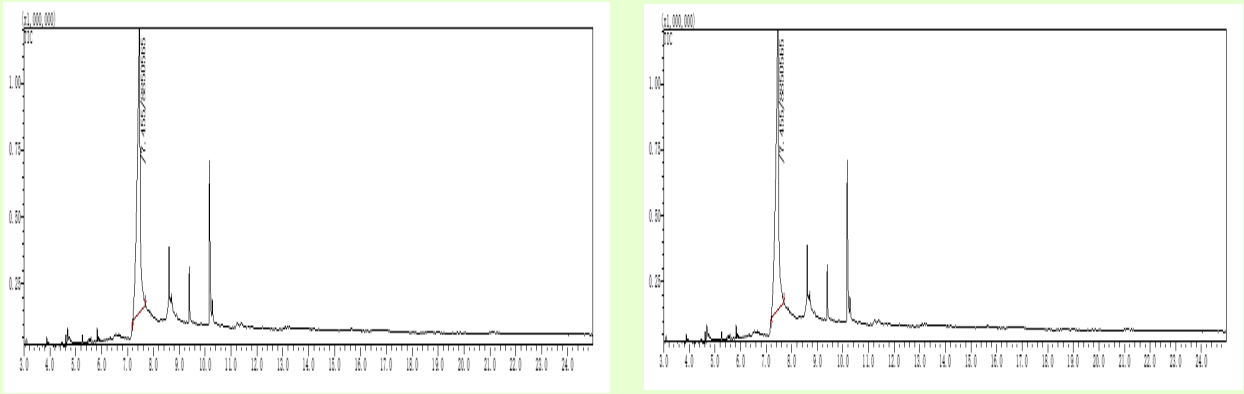


Figure: 10 GC-MS total ion flow diagram (left) and mass spectra (right) of phenylalanine.

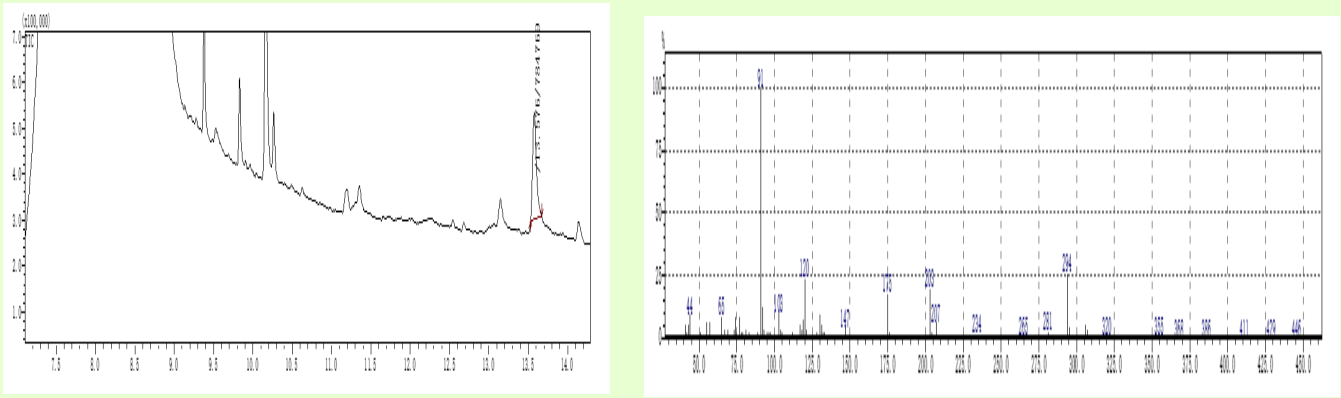


Figure 11: GC-MS total ion flow diagram (left) and mass spectra (right) of Cyclo(L-Phe-Phe) by heating phenylalanine.

Conclusion

In this paper, the influence of the composition of paper and various components in sweat on the fluorescence effect is studied. Conclusions are obtained that the composition of paper and various components in the sweat do not affect the heat induced fluorescence reaction. Only amino acids can be heated to produce the fluorescence effect. The fluorescent substance is generated by the reaction of amino acid in sweat heating, and the higher concentration of amino acids, the better effect of fluorescence. The experiments also verify that amino acids can produce fluorescent substance in the absence of paper.

The fluorescent substance is mainly generated by the heating reaction of amino acids in human sweat. The infrared spectrum, GC-MS and nuclear magnetic resonance methods are used to verify the experimental conclusions that are deduced theoretically. It is concluded that two molecules of the same kind of amino acids in a certain temperature environment generated a rigid heterocyclic fluorescent product of a six element ring through dehydration condensation. As shown in Figure 12, 13 and 14 in the experiment, the heat induced fluorescence reaction equation of glycine, alanine and phenylalanine was obtained. They respectively generated 2,5-piperazinedione, 3,6-dimethylpiperazine-2,5-dione, and Cyclo (L-Phe-Phe) by reaction.

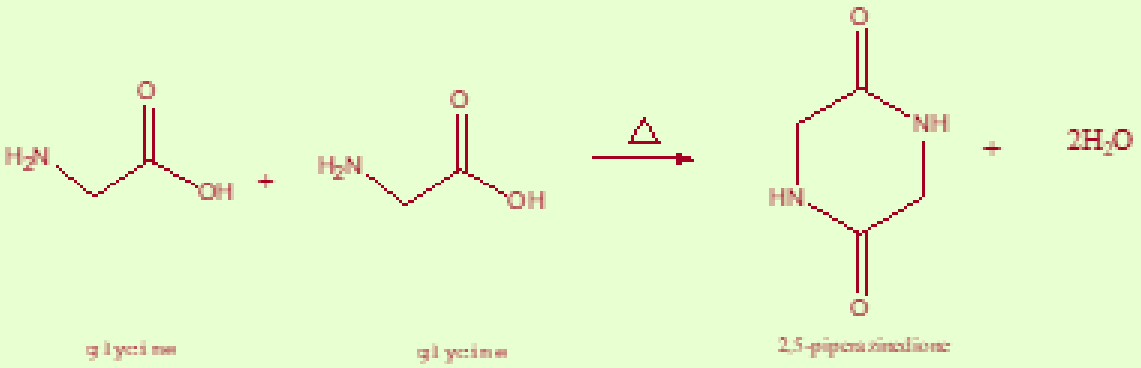


Figure 12: The heat induced fluorescence reaction equation of glycine.

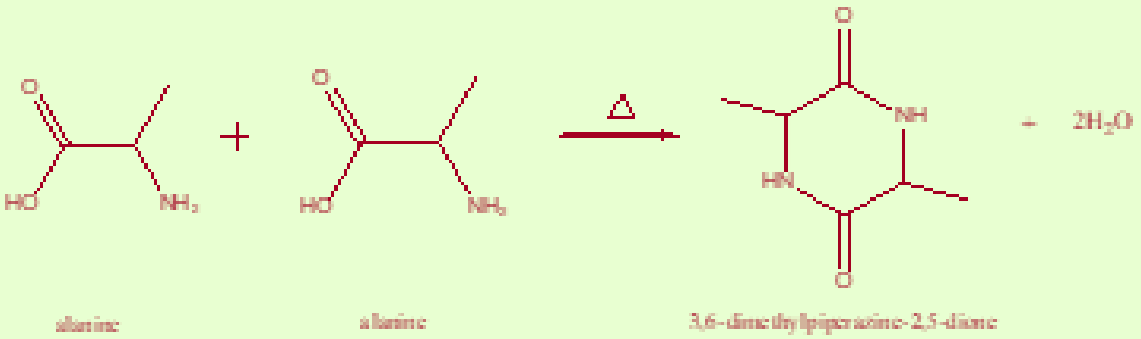


Figure 13: The heat induced fluorescence reaction equation of alanine.

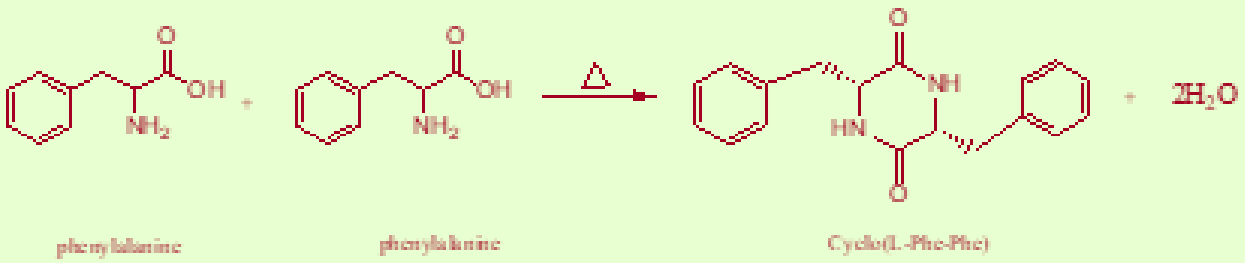


Figure 14: The heat induced fluorescence reaction equation of phenylalanine.

Reaction mechanism of the heat induced fluorescence imaging technology is not only the fluorescence reaction of the three amino acids. There may be a similar heat induced fluorescence reaction process between two molecules of the same kind of other amino acids and different amino acids in sweat. All these have very good research meaning and research value, so further experiments and studies are needed to confirm these conclusions.

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Identification and characterization of a new synthetic cathinone, 4′-fluoro-α-pyrrolidinovalerophenone (4F-PVP), in China

Changying[1,], Zhaoyang[1], Hu Yupeng[2], Yuan Mingjun[3], Hukun[3], Huangxing[1], Gao Li-sheng[1]*
[1.] Institute of Forensic Science, Ministry of Public Security, People's Republic of China
[2.] Public Security Bureau of Huizhou city, People's Republic of China
[3.] Material Evidence Authentication and Research Center of DeZhou Plublic Security Bureau, People's Republic of China

Abstract

New psychoactive substances (NPS) have gained much popularity on the illicit market over the past years. A new synthetic cathinone, 4′-fluoro-α-pyrrolidinovalerophenone was identified in powder from a tablet seized by the local police in China. The identification was based on liquid chromatography-high resolution mass spectrometry (LC-HRMS), gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy. To our knowledge, no chemical or pharmacological data about this compound has appeared until now, making this the first report on the compound.

Introduction

New psychoactive substances (NPS) are a serious threat throughout the world. According to the world drug report 2018, between 2009 and 2017, a total of 803 NPS had been reported by 111 countries and territories to the UNODC early warning advisory on NPS. Despite that, there are still many NPS that are not being monitored, and therefore their health effects are not yet studied. Hence, many cases of intoxication and death related to NPS have been reported in the past few years, highlighting the public health risks of these substances [1].

Forensic laboratories, universities, research institutes and law enforcement agencies play an important role in monitoring these types of substances, which are also encountered in customs seizures and medical emergencies. The aim of the work was the determination of the main compound present in a seized tablet. The structure of a new substance was elucidated by using various analytical techniques. Characterization of the molecule was performed by GC–MS, LC-HRMS and NMR. Current information reported in this article will be useful for other laboratories in order to monitor the presence of this novel NPS or other related compounds in seizures and biological samples.

Experimental

Sample

The sample, a white tablet, was submitted by the local police. According to the information provided by the consumer, it was purchased from the Internet, but no information was received about the consumption or psychoactive effects of this product.

Experimental Condition

For GC–MS analysis, an Agilent 7890A gas chromatograph was coupled to a 5975C mass spectrometer detector (Agilent, USA). The oven temperature was initially maintained at 60°C and programmed to reach 280°C at 15°C/min, and maintained for 15 min (total run time was 30 min).

LC-HRMS analysis was performed using a Shimadzu (Kyoto, Japan) HPLC system. Separation was performed with a Poroshell 120 EC-C18 column (5 cm× 3.0mm, 2.7 μm). For gradient elution, the mobile phases were 0.1% formic acid in water (A) and acetonitrile (B) and were mixed according to the following conditions: 0–0.5min, 5% B, linear to 90% B at 6min, held at 90% to 8min, returned to 5% B at 8.1min and equilibration to 12min. The flow rate was 0.4mL/min and the injection volume was 1 μL.

The NMR spectra were obtained on an Avance III 400 spectrometer (Bruker, Bremen, Germany) at 300 K with 400MHz for ^{1}H and 100 MHz for ^{13}C NMR. Assignments were made via ^{1}H–NMR, ^{13}C–NMR, ^{13}C–distortionless enhancement by polarization transfer (^{13}C–DEPT), ^{1}H/^{1}H correlation spectroscopy (^{1}H/^{1}H–COSY), ^{1}H/^{13}C–heteronuclear single-quantum correlation spectroscopy (^{1}H/^{13}C–HSQC), and ^{1}H/^{13}C–hetero nuclear multiple-bond correlation spectroscopy (^{1}H/^{13}C–HMBC).

Sample Treatment

For GC–MS analysis, 10 mg of sample was extracted with 10 mL of MeOH assisted with sonication during 15 min. Finally, the extract was centrifuged to remove insoluble material and afterwards directly injected into the GC–MS system.

For LC-HRMS analysis, 10 mg of sample was weighed in 15 mL glass tube and extracted with 10 mL of Acetonitrile in an ultrasonic bath for 15 min. After centrifugation at 10 000 rpm during 10 min, the supernatant was diluted 1000-fold with mobile phase, and 1 μL of the extract was injected in the LC-HRMS system, using MSE acquisition mode.

For NMR analysis, 10 mg of sample was extracted in 0.6 mL of MeOH-d_4.

Results and Discussion

Gas chromatography–mass spectrometry

Firstly, sample was analyzed by GC–MS. The total ion chromatogram showed an intense and single peak at 10.712min. The application of NIST spectral library did not retrieve results. At this point, the interpretation of fragment ion in EI spectra was performed. The EI spectrum of the chromatographic peak at 10.712 min showed four intense m/z ions (Figure 1). Additional fragment ions were observed at m/z 95, 55 and 75, the fragment ion at m/z 126 being the base peak. Thus, additional analytical techniques were necessary for the elucidation of the structure.

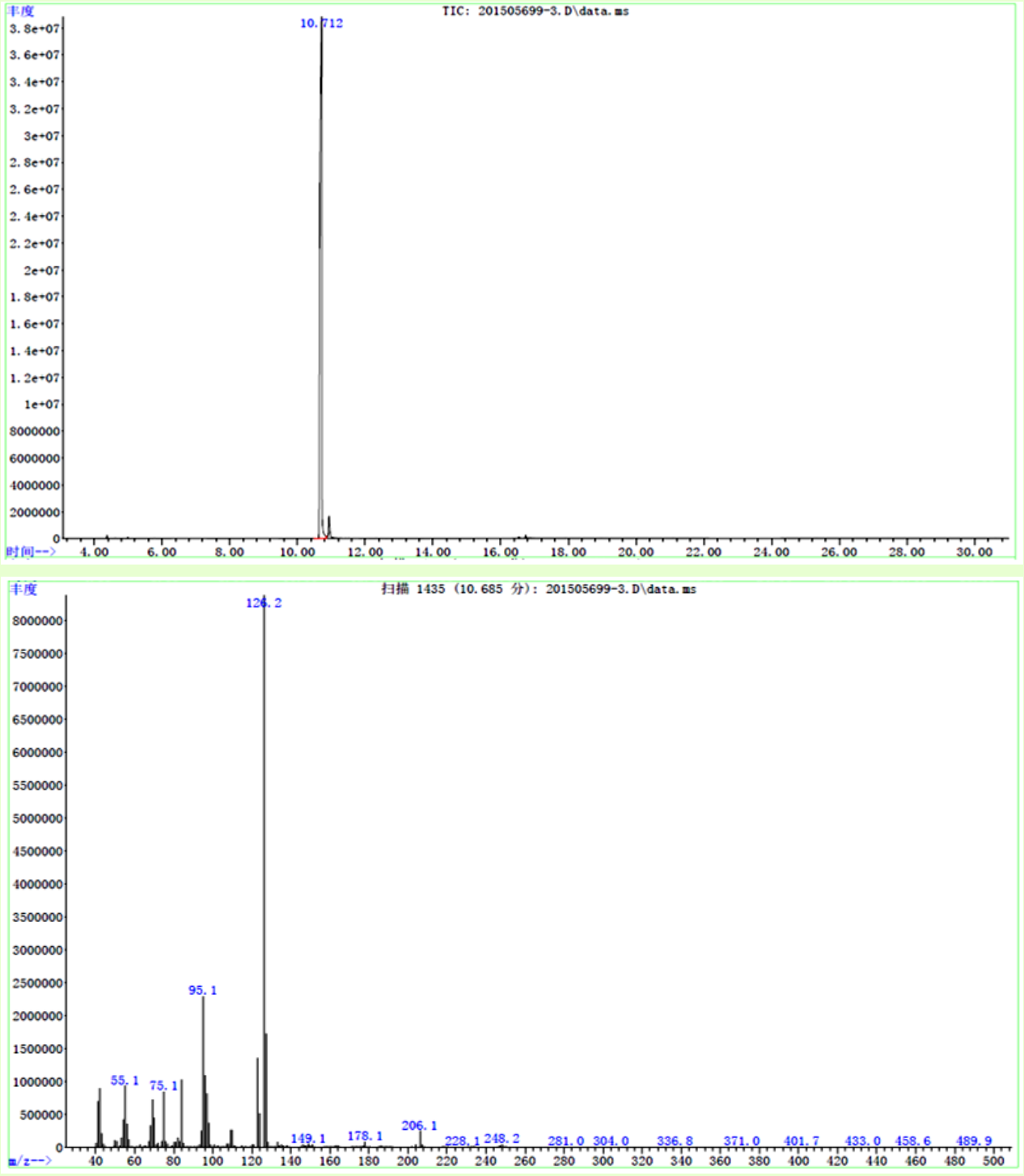
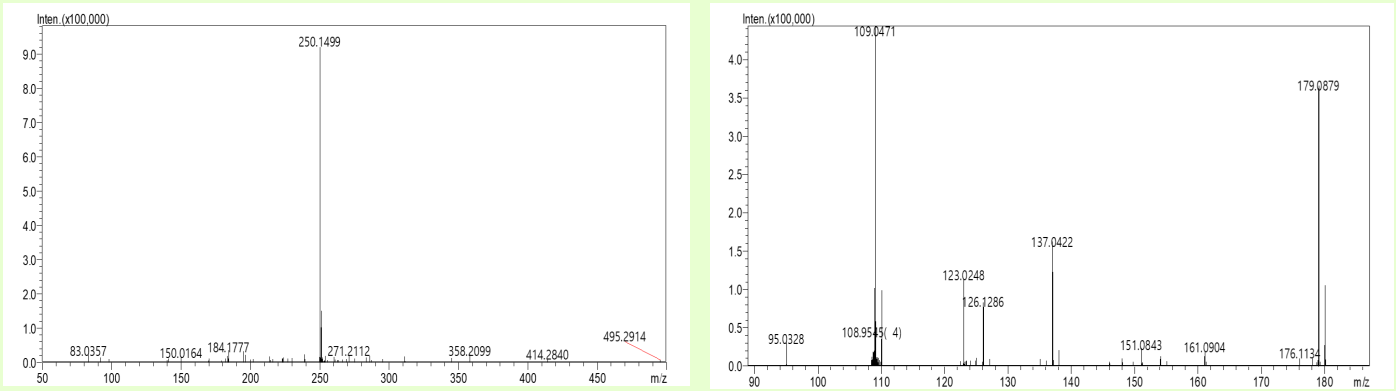


Figure 1: EI mass spectrum of the unknown compound.

Liquid chromatography – high-resolution mass spectrometry

Under the gradient program used in LC-ESI-QTOF -MS analysis, the investigated compound eluted at the retention time of 2.98 min. Protonated ions for the unknown compound was observed in full scan MS^1 mode (Figure 2A), from which the accurate masses and chemical formulae was determined. The fragmentation of the molecular ions was performed in the MS^2 mode (Figure 2B). Exact and accurate masses as well as the chemical formula predicted for main product ions observed are summarized in Table 1, while its fragmentation routes following ESI ionization are proposed in Figure 3.



(A) (B)

Figure 2. Mass spectra of the unknown compound obtained by LC-ESI-QTOF-MS in the full scan MS^1 mode (A) and the MS^2 mode (B)

RT(min)	Chemical Formula	Exact Mass	Accurate Mass
2.98	$C_{15}H_{21}FNO^+$	250.1607	250.1499
	$C_7H_6F^+$	109.0454	109.0471
	$C_{11}H_{12}FO^+$	179.0872	179.0879
	$C_8H_6FO^+$	137.0403	137.0422
	$C_7H_4FO^+$	123.0246	123.0248
	$C_8H_{16}N^+$	126.1283	126.1286
	$C_6H_4F^+$	95.0297	95.0328

Table 1: Accurate masses of the protonated molecules and product ions and its proposed chemical formulae obtained for the unknown compound measured by LC-ESI -QTOF-MS.

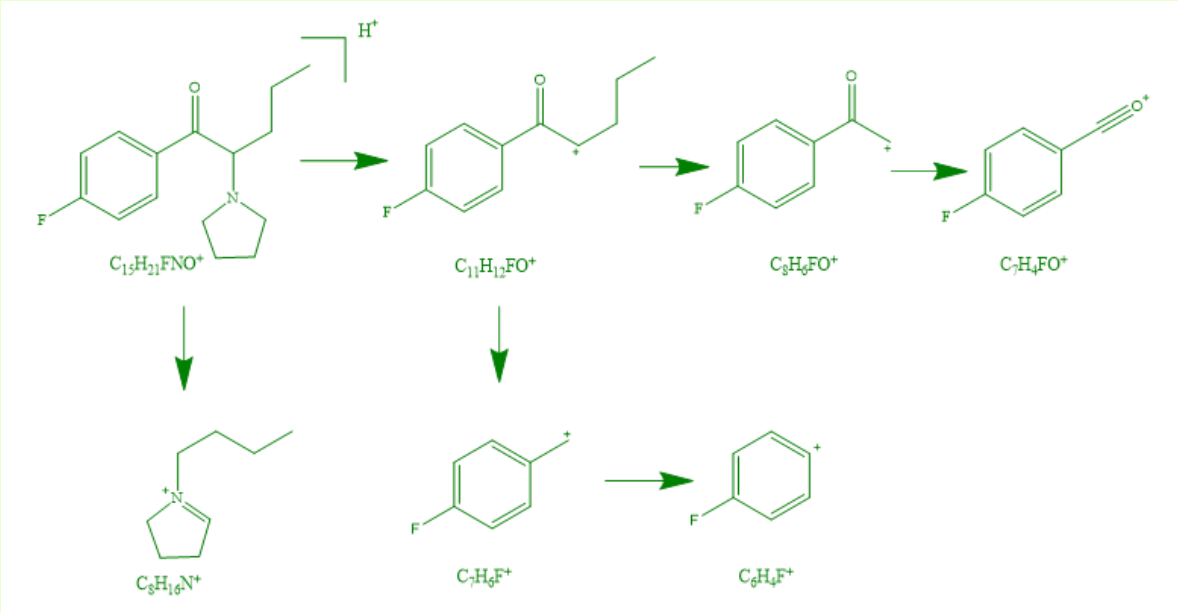
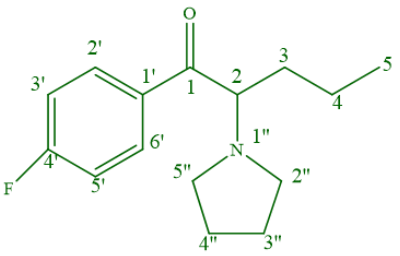


Figure 3: Proposed fragmentation routes of the protonated molecule and product ions of the unknown compound by LC-ESI-QTOF-MS.

Nuclear magnetic resonance

The structure of the known compound was further elucidated by NMR analysis (Table 2). The NMR data from position 1' to 6' suggested the presence of the benzene ring moiety. The chemical shifts of the carbons from C-1 to C-5, and from C-2" to C-5" of the compound were also in agreement with the previously reported identical side chain of α-PVP[2], which indicated that this compound had a similar 2-(pyrrolidin-1-yl)pentan-1-one moiety. Because of the presence of F atom, the nuclear fission of C-4'' has two peaks, and the hydrogen atoms in the benzene ring were fissioned into two groups.



	^{13}C(δ/ppm)	^{1}H (δ/ppm, protons, multiplicity[c], coupling constants) ^{1}H/^{13}C–HSQC correlation	^{1}H/^{1}H–COSY correlation	^{1}H/^{13}C–HMBC correlation
1	195.86			
2	70.34	5.40,1H,dd	3	1,3,4,2",5"
3	33.53	2.04,2H,m	2,4	2,4,5
4	18.48	1.24,2H,m	3,5	2,3,5
5	14.17	0.88,3H,t	4	3,4
1'	132.24;132.21			
2'	133.35;133.28	8.20,1H,m	3'	1,4',6'
3'	117.71;117.53	7.36,1H,m	2'	1',4',5'
4'	169.40;167.35		5'	1',3',5'
5'	117.71;117.53	7.36,1H,m	4'	
6'	169.40;167.35	8.20,1H,m		1,2',4'
2"	53.28	3.71,1H,m;3.38,1H,m	3"	3"
3"	24.12	2.24,1H,m;2.08,1H,m	2",4"	
4"	24.21	2.15,1H,m;2.08,1H,m	3",5"	
5"	56.32	3.67,1H,m;3.09,1H,m	4"	4"

Table 2. ^{1}H and ^{13}C NMR and diagnostic correlations in two-dimensional spectra for the unknown compound.

Conclusions

In this work, we identified 4´-fluoro-α-pyrrolidinovalerophenone in a tablet, and this is the first report about 4F-PVP in China. The complete identification of the compound required the combination of different analytical techniques, such as GC–MS, LC-HRMS and NMR. The fragmentation pathway of this compound in LC-HRMS has also been proposed, in order to make easier the future identification of related compounds by common fragmentation analysis. Unfortunately, the psychoactive effects and toxicity have not been evaluated yet. The strategy applied in this work has proven to be a powerful workflow for the identification and characterization of novel NPS. The information obtained about this new compound will be useful for forensic laboratories or to enhance early warning systems.

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QuEChERS sample preparation combined with liquid chromatography-tandem mass spectrometry for the determination of carbamate pesticides in human whole blood

Mr. Wan Chaochao[1], Dr. Ren Xinxin[2], Prof. He Hongyuan[1], Mrs. Wang Aihua[2], Dr. Cui Guanfeng[2], Mr. Song Ge[2], Mr. Dong Linpei[2]*
[1.] People's Public Security University of China，People's Republic of China
[2.] Institute of Forensic Science, Ministry of Public Security，People's Republic of China
(the corresponding author)*

Abstract

A modified QuEChERS extraction method followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been developed for the determination of five carbamate pesticides in human whole blood. The important parameters in QuEChERS method, such as extraction solvents, salts, and cleaning reagents, are optimized. It is suggested by the experiment that the method is user-friendly and rapid with high recovery and low matrix interference.

Introduction

QuEChERS (quick, easy, cheap, effective, rugged and safe) firstly reported by Anastassiades et al. in 2003[1] is a sample preparation technique based on principles of liquid-liquid extraction[2] and dispersive solid-phase extraction[3]. It has the advantages of being easy, effective, and rugged compared with the conventional methods, such as liquid-liquid extraction and solid-phase extraction. The QuEChERS method has been extensively used for analysis of pesticide residue[4], veterinary drug residue[5] and fungal toxin in produce[6], however a few studies have utilized this technique for biological samples[7]. In this study, we modified the QuEChERS method for the extraction of five carbamate pesticides (Methomyl, Metolcarb, Propoxur, Carbaryl and Isoprocarb) in human whole blood. By coupling with LC-MS/MS, a rapid, simple and effective method was established.

Materials and Methods

Chemicals and reagents

Acetonitrile, methanol, formic acid and ammonium acetate were HPLC grade (Fisher Scientific, USA). Methomyl, metolcarb, propoxur, carbaryl, isoprocarb, and methomyl-D3 were purchased from Dr. Ehrenstorfer (Germany). Methomyl-D3 was used as an internal standard (IS). PSA (primary secondary amine) and Bondesil-C18 were purchased from Agela Technologies (China) and Agilent Technologies (USA), respectively.

Sample Preparation

10 μL IS at a concentration of 1 mg/L was added to 1.0 mL blood sample, and 2 mL acetonitrile and 30 mg NaCl were added. The mixture was then shaken for 5 min and centrifuged at a rate of 8000 r/min for 5 min. The supernatant liquid was transferred into a plastic centrifuge tube with 150 mg anhydrous MgSO4, 25 mg PSA and 25 mg C18. The mixture was vortexed for 15 s and centrifuged for 5 min. Finally, the supernatant was analyzed by LC-MS/MS.

Instrumentation

Separation was performed with a Shimadzu LC-30AD coupled to an AB SCIEX QTRAP 5500 system. Sample volume of 1 μL was injected onto a ZORBAX Eclipse Plus C18 column (2.1×150 mm, 1.8 μm). A gradient elution was conducted by using water with 5 mmol ammonium acetate-0.1% formic acid (mobile phase A) and methanol (mobile phase B) at a flow rate of 0.4 mL/min, as shown in Table 1.

Time（min）	A（%）	B（%）
0.20	90	10
4.00	10	90
5.00	10	90
5.10	90	10
5.50	90	10

Table 1: Gradient profile

The mass spectrometer with electrospray ionization source was operated in multiple reaction monitoring (MRM) mode in positive ion mode. The MRM conditions of five carbamate pesticides and the IS are listed in Table 2.

Results and Discussion

Optimization of QuEChERS method

Choice of Solvent:

Compared with methanol and ethyl acetate, acetonitrile exhibited higher extraction efficiency, and when the volume proportion of blood to acetonitrile was 1:2, the best protein precipitation effect was realized.

Compound	Parent ion (m/z)	Product ion (m/z)	Declustering potential (V)	Collision energy (eV)
Methomyl	162.9	88.0*	40	12
		106.1	40	13
Metolcarb	166.0	109.1*	40	15
		94.1	40	40
Propoxur	210.0	168.0*	40	10
		153.1	40	10
Carbaryl	202.1	145.1*	40	14
		127.1	40	37
Isoprocarb	194.1	137.0*	45	11
		152.1	45	10
Methomyl-D3 (IS)	165.9	88.0*	40	12
		105.9	40	14

** Quantitative ion*

Table 2: Mass spectrometric parameters of carbamate pesticides.

Salting-out Steps:

NaCl was added to decrease the solubility of pesticides in the blood and to increase their concentration in acetonitrile. When 30 mg NaCl was added, the recoveries of the five carbamate pesticides were the highest.

Sample Clean-up:

Currently, frequently used cleaning agents in QuEChERS mainly involve C18, PSA. Besides, anhydrous MgSO4 is also added to remove redundant water. After optimization, we chose the combination of 150 mg MgSO4 + 25 mg PSA + 25 mg C18, which can effectively reduce matrix effects and realize the 88.9%-92.6% recovery of the 5 carbamate pesticides.

Figure 1 shows the LC-MS/MS spectra obtained from a whole blood sample spiked at 10 μg/L, which was conducted upon the optimized sample preparation and LC-MS/MS parameters.

Method Validation

Blank blood samples were spiked at six different concentration levels and then analyzed. The 5 carbamate pesticides show good linear relations within the concentration range of 1-100 μg/L, correlation coefficients *(r)* >0.9963, LOD is 0.5 μg/L and LOQ is 1 μg/L. In blank blood samples, standard solution of 1, 10 and 50 μg/L were added respectively for 6 parallel spiked experiments, which suggested average recoveries of 88.6%-102.6% and RSDs of 2.8% -7.9%.

Conclusion

A QuEChERS-LC-MS/MS method for simultaneous detection of five carbamate pesticides in human whole blood was established. The optimized QuEChERS pretreatment method integrates quickness, convenience and simplicity of protein precipitation and low matrix effects of solid-phase extraction, effectively reduces matrix effects, guarantees a relatively high recovery rate, and meets the demands in actual cases.

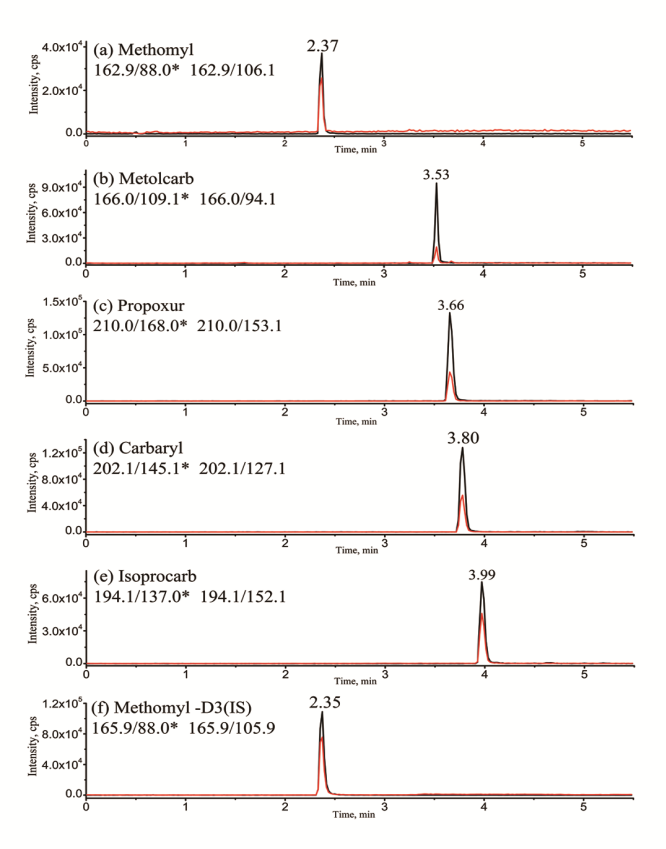

Figure 1: LC-MS/MS spectra obtained from a whole blood sample spiked at 10 μg/L 5 carbamate pesticides solutions.

Acknowledgement

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Case Study

Validation of DNA Mixture Protocols: How Many is Enough?

Dr. Maria Corazon A. De Ungria, PhD
University of the Philippines Diliman Quezon City, Philippines

The urgent need to address cross-border crimes in the Asia-Pacific region pushed six nations, namely, Brunei, Indonesia Malaysia, Philippines, Singapore and Thailand to form the Asian DNA Profiling Group (ADNAP) in 2007. In 2008, the ADNAP was renamed the DNA Working Group (DNA-WG) when it was integrated into the newly founded Asian Forensic Science Network (AFSN). Since the ADNAP days to the present, the members of the DNA-WG had been discussing the value of sharing common protocols in order to increase the effectiveness of forensic investigations within the entire region, regardless of political, economic and cultural differences. In 2014, the Health Science Authority (HSA) of Singapore initiated the first DNA Proficiency Exercise and invited member institutes to participate by genotyping unknown reference samples. From 2015 to the present, HSA focused on proficiency exercises involving DNA mixtures, highlighting the challenges that member institutes faced in handling and analyzing this type of sample. Evaluation of the three proficiency tests showed differences in genotyping results and data interpretation among participating laboratories. The sources of these differences were not discussed during the business meetings but did raise concerns regarding the need to look more closely into how laboratories validated their DNA mixture protocols before these were used in forensic casework. Could variations in the DNA mixture validation protocols of participating laboratories partly explain the differences in the DNA profiling results of the proficiency tests? Because of the need to generate accurate genotypes for the purpose of identifying the individual contributors in a DNA mixture, validation protocols must test all parameters that impact mixture interpretation such as sensitivity, specificity, repeatability, reproducibility, linearity and quality of profiles (1).

In 2018, whilst designing an internal laboratory validation study on two- person DNA mixtures in order to compare results from capillary-based analysis and Next Generation Sequencing (NGS) technology, research scientists of the DNA Analysis Laboratory, University of the Philippines Diliman- Natural Sciences Research Institute realized that the number of biological and technical replicates required for DNA mixture validation was not uniform in many published papers. Wary of falling into the danger of “over-validation” that would result in the misuse of human and material resources and would delay laboratory operations (2), an informal clarificatory query was sent to eleven forensic scientists from ten countries to determine how established forensic DNA laboratories conducted their internal validation for DNA mixtures. Surprisingly, the responses were not straight-forward and showed the underlying variation in the appreciation of the requirements to demonstrate repeatability of DNA mixture validation results. Some laboratories used the word ‘replicate’ to simply mean the number of times a reaction was performed, without differentiating a biological replicate from a technical replicate. A few others defined replicate as the number of contributors in a mixture. Because of these responses, the need to differentiate biological and technical replicates in order to better address the intended use of both types of replicates in internal validation studies is warranted.

Repeatability is an integral component of science because replication provides reliable estimates of the typical variability derived from using a method (3). Biological replicates are used to evaluate the sources of random biological variation whereas technical replicates provide information on the variability derived from repeated measurements of the same sample. Simply stated, biological replicates consist of different samples that share common properties with respect to the variable being measured and are expected to be very similar (but not identical) with regard to the test. Increasing the number of biological replicates improves the efficiency of statistical testing (4). In fact, the Eurachem Guide recommends the use of 6-15 replicates for each material to be tested by one analyst using the same equipment in the same laboratory and performed during a short timescale (3). Technical replicates, on the other hand, can provide an assessment of the variation resulting from the use of an equipment or the application of a protocol on the same sample. Technical replicates are useful in limiting the impact of measurement error (4).

In applying the concept of biological and technical replicates to DNA mixtures studies where the analysis focuses on more than one source of DNA, a two-person mixture combination, e.g. source 1-source 2, should be considered as a ‘sample’ or biological replicate. The type of mixture e.g. male-female, male-male may also be considered separately depending on the type of DNA markers, e.g. Y-chromosomal STR vs autosomal STR, tested. In the US, the Scientific Working Group on DNA Analysis Methods (SWGDAM) released the “Validation Guidelines for DNA Analysis Methods” to supersede an earlier set of standards published in 2010 (5). The 2016 document put forward the importance of establishing guidelines for DNA mixture interpretation in order to obtain reliable results. However, both the 2010 (6) and 2016 SWGDAM Guidelines left the determination of the number of biological and technical replicates to the laboratory

conducting the validation. In a draft document released by the Academy Standards Board of the American Academy of Forensic Sciences (ASB-AAFS) for mixture studies, the requirement to conduct internal validation studies on all procedures used for mixture interpretation was also highlighted (7). Like SWGDAM, ASB-AAFS did not provide explicit instructions on how forensic DNA laboratories, many of which do not have experts in statistics, would determine the number of biological and technical replicates needed to ensure the statistical robustness of their internal validation results. Notably, validation studies should provide the information needed to calculate thresholds, e.g. analytical, stochastic and stutter, and estimate true peak ratios. These parameters are important in interpreting DNA mixture data in actual casework, where the number of contributors and actual genotypes are unknown, thus making the analysis more complex (8).

Unlike SWGDAM and ASB-AAFS, the European Network of Forensic Science Institutes (ENFSI) prescribed the use of five to ten samples for the validation of a specific parameter to demonstrate repeatability of forensic protocols (1). Notably, this range overlaps with the Eurachem Guide of 6-15 replicates for the validation of any method (3). Applying the ENFSI recommendations in male-female DNA mixture analysis will result in the use of five to ten different male-female combinations. The ENFSI Guidelines further require DNA mixture validations to test a 'series of different laboratory defined mixture ratios in triplicates'. Hence, validation for a male-female mixture using four ratios in a dilution series for five to ten replicates amplified three times per dilution would result in 60 to 120 amplification reactions. Because of the workload of most forensic DNA laboratories, it is reasonable to recommend the adoption of the minimum requirement of five male-female combinations with four dilutions each, resulting in 60 amplifications for the conduct of two-person DNA mixture validations.

Using the accumulated knowledge and expertise in forensic DNA testing, we have opted here to answer the question of "How many is enough?". We also take a step further by recommending the formulation of detailed and uniform DNA mixture validation procedures for member institutes of the AFSN DNA-WG in order to bring us closer to our goal of sharing common protocols and expertise in the Asia-Pacific region.

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Author's email: madeungria@up.edu.ph

Forensic DNA methylation-based body Fluids Identification of casework samples

*Ji-Eun OH, Chohee Kim, Min-Hee Kim, Sung-Jin Lee, So Yeun Kwon, Chong Min Choung, Pil Won Kang, Si-Keun Lim**
National Forensic Service, Republic of Korea

The serological tests of the biological evidence from crime scene enable to characterize the body fluids such as blood, saliva and semen, and provide key clues to solving the crime cases with followed DNA analysis. But the serological tests have some limitations. There is a possibility of false positive result, and some body fluid (e.g. vaginal fluid and menstrual blood) can't be identified by serological tests. It is also difficult to characterize the mixture of different types of body fluid, a small amount of the samples and when we have only extracted DNA.

Recently the DNA methylation analysis has been suggested as a solution of these limitations. It enables to identify the body fluids from DNA using the selected CpG markers which are specifically methylated in each body fluid type.

In the previous study, the DNA methylation analysis method using the Multiplex SNaPshot system has been developed and could identify 5 types of body fluid such as blood, saliva, semen, vaginal fluid and menstrual blood simultaneously [1]. The multiplex PCR was performed using bisulfite converted DNA with 9 CpG markers composed of selected 1 to 2 body fluids specific CpG markers. Then single-base extension was performed with dye-labeled ddNTPs, and the fluorescence signals were detected by the Capillary Electrophoresis.

The limit of detection (LOD) value was produced to 1.25ng/μL for blood, 0.625ng/μL for semen and 0.315ng/μL for saliva.

In this study, we collected various casework samples and performed the DNA methylation analysis using the Multiplex SNaPshot system with body fluid-specific markers reported by Lee et al. [1] to confirm its feasibility to forensic evidences. In addition, we compared the results from DNA methylation analysis with the serological test results.

Case 1 was a sexual assault case. The cotton swab of victim's abdominal region was negative for both of semen and saliva tests. STR profile of the suspect was produced only in sperm fraction, and only the victim's STR profile was in epithelial fraction. The DNA methylation analysis identified that the suspect's profile was derived from semen (Figure 1). There was no serological test to identify vaginal fluid, so serological test was not needed for the suspect's penis swabs. However the victim's STR profile was detected in the suspect's penis sample and the DNA methylation analysis identified that this profile was derived from victim's vaginal fluid.

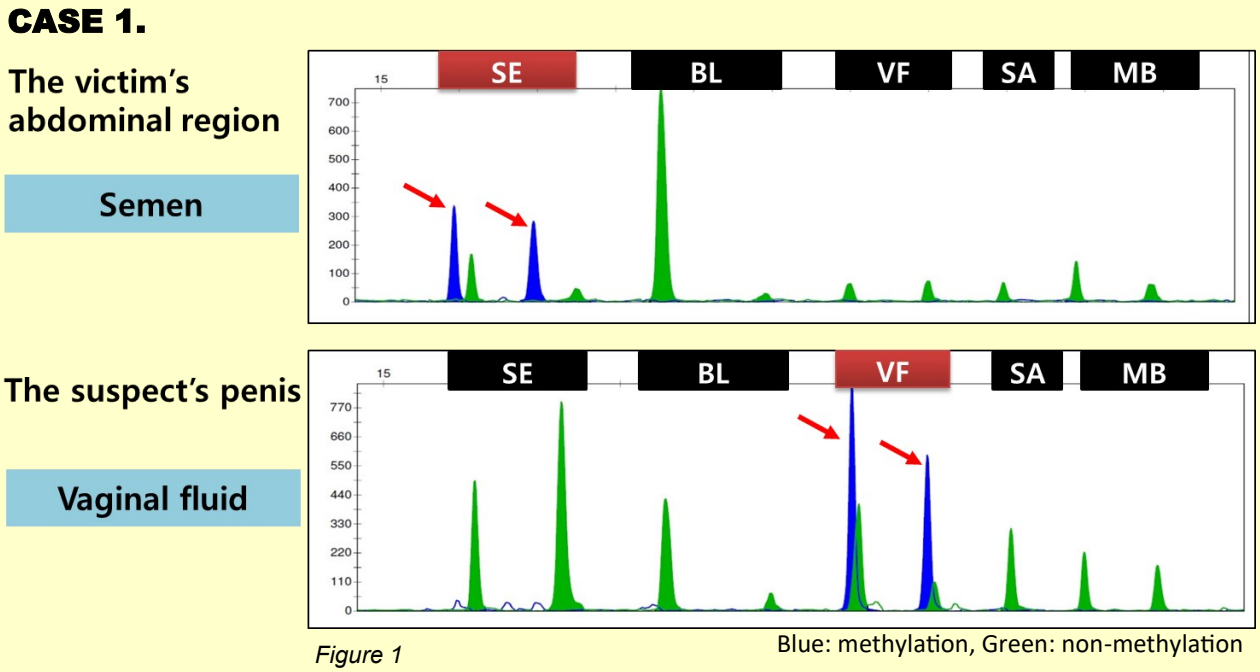

Figure 1 Blue: methylation, Green: non-methylation

Case 2 is a blackmail case. Despite the swabs of the buttons of public phone were considered as touch evidence at first, the DNA concentration was much higher than the expected value and showed a clear single man's profile. The DNA methylation analysis revealed the DNA was derived from blood (Figure 2).

Case 3 is a murder and arson case. A dead body that had a stab wound at neck was found at the scene of fire. Blood-stained toilet papers were found near the victim. It was positive for blood, but was negative for semen. The victim's profile was detected by STR typing and the DNA methylation analysis confirmed that the blood stains on toilet papers was menstrual blood (Figure 3).

CASE 2.

The buttons of public phone

Blood

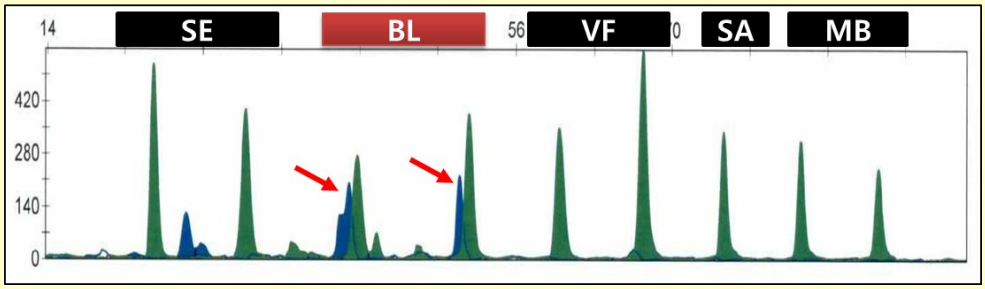

Figure 2 Blue: methylation, Green: non-methylation

CASE 3.

The toilet papers

Blood
Vaginal fluid
Menstrual blood

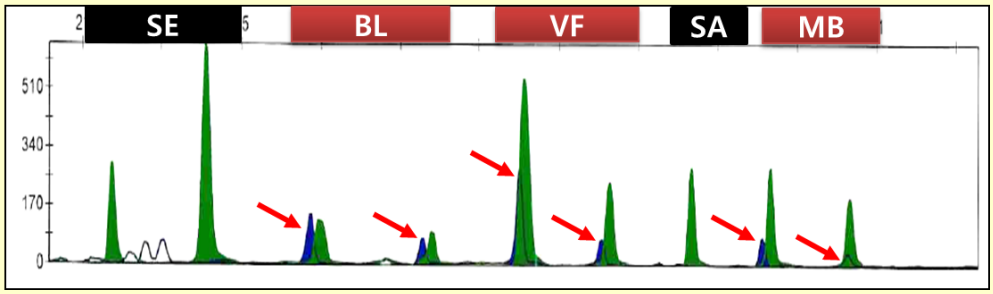

Figure 3 Blue: methylation, Green: non-methylation

Case 4 is a murder case and a knife and an unknown stain were found near the victim. Despite LMG test for blood was negative, the victim's STR profile was detected on the blade of knife. The DNA methylation analysis confirmed that the victim's DNA was originated from vaginal fluid. Since her left nipple and pudendum were cut, this result agreed with the situation. The unknown stain was positive for saliva and showed a man's profile. The methylation analysis identified as saliva which was concordant to the serological test of SALIgAE® for saliva (Figure 4).

In conclusion, The DNA methylation-based body fluids identification method was successfully applied to identify five different types of body fluid (blood, saliva, semen, vaginal fluid and menstrual blood). Firstly, DNA methylation analysis enabled to identify diverse type of body fluid with a small amount of sample at the same time. Secondly, it could be possible to identify vaginal fluid and menstrual blood of which no serological tests. Thirdly, DNA methylation analysis could confirm whether DNA was contaminated and find out the source of contamination. Lastly, in sexual assault cases with mixed body fluid evidences, DNA methylation analysis was helpful to infer the crime details or the suspect's various actions that victim can't remember. In conclusion, we suggest that DNA methylation-based body fluid identification can provide the court decision with a critical clue and be a useful tool for solving crimes.

Reference

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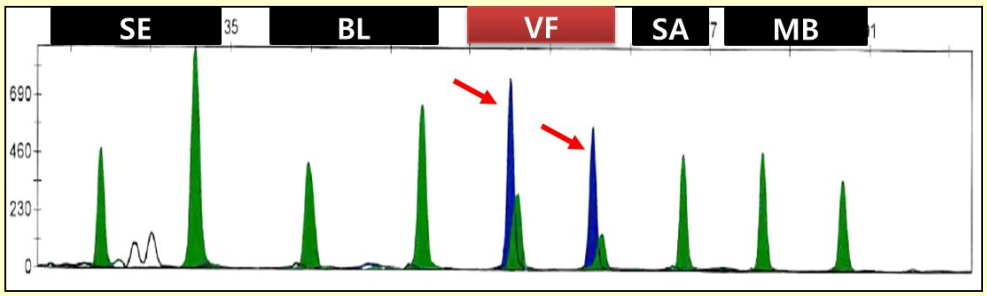
CASE 4.

The blade of knife

Vaginal fluid



The unknown stain

Saliva

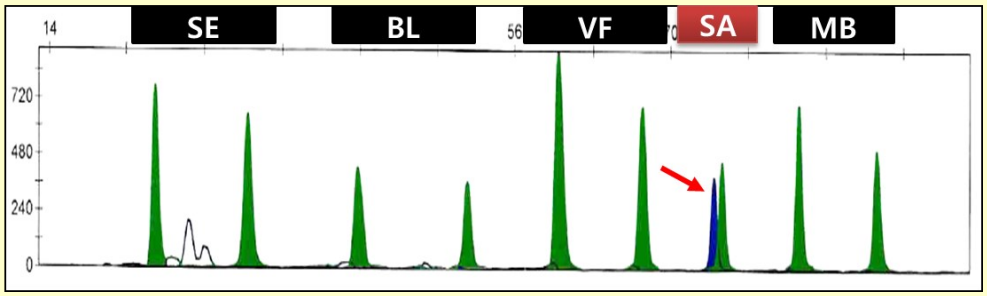


Figure 4 Blue: methylation, Green: non-methylation

AFSN Member Institutes

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2	Department of Scientific Services, Brunei Darussalam	**28**	Institute of Forensic Science, Shandong Public Security Department, People's Republic of China
3	Centre for DNA Fingerprinting and Diagnostics, India	**29**	Institute of Forensic Science, Suzhou Public Security Bureau, People's Republic of China
4	Directorate of Forensic Science, Himachal Pradesh, India	**30**	Institute of Forensic Science, Tianjin Public Security Bureau, People's Republic of China
5	Department of Police Medicine of the Indonesian National Police, Indonesia	**31**	The Institute of Evidence Law and Forensic Science, China University of Political Science and Law, People's Republic of China
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12	Korea Coast Guard Research Institute, Korea	**38**	Government Analyst's Department, Sri Lanka
13	National Digital Forensic Center of Supreme Prosecutors' Office, Korea	**39**	National Dangerous Drugs Control Board, Sri Lanka
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(As of 21 June 2018)