ForensicAsia
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# AFSN President's Address

Dear colleagues and friends,

We have passed the mid-point of 2021. In the past few months, the COVID-19 situation is constantly evolving and many countries are facing continuous challenges in tackling the spread of the virus and its various variants. Despite all these, the AFSN Board is firmly committed to our goal of providing a forum for forensic labs in Asia to discuss on issues relating to forensic science and enhance the quality of forensic services through expert working groups, sharing and training. I would like to take this opportunity to thank Pol. Lt. Col. Wannapong Kotcharag, Central Institute of Forensic Science, Thailand, for his past contribution to the AFSN and who have since stepped down as an AFSN Board Member. At the same time, I warmly welcome Pol. Col. Songsak Raksaksakul, Director-General of the Central Institute of Forensic Science, as a new Board Member to AFSN.

***Board Meetings***

The Board has met twice virtually on the 2nd of February and 4th of June to discuss and plan the activities for the network. We have evaluated the feedback gathered after last year's virtual AGM so as to understand members' needs and improve on the content and format of the upcoming meeting. We are very happy to see many positive feedback from participants coming from 17 member institutes of 11 countries, and the suggestions that you have given. The Board has decided that moving forward, the workgroups and committee will issue certificates to all speakers and presenters of scientific papers at the Annual Meeting to recognise the contributions made as well as to encourage more participation amongst the experts from our member institutes.

***Workgroup/Committee Activities***

The Board held a special meeting with all workgroup/committee office bearers on the 26th of February to communicate the Board's strategy for the year, as well as the planned activities for 2021-2022. It was a fruitful discussion where all workgroups/committee were committed to organising two activities annually as far as possible, one in the first half year and the second during the Annual Meeting & Symposium, so as to keep our experts engaged in ongoing discussion and sharing. There will be a series of workshops organised by the various workgroups/committee from June to August.


A total of 31 participants joined the discussion with AFSN President Dr Angeline Yap. This include Chairs of CSIWG, FPWG, IDWG, TEWG, TXWG and QASC and representatives of all WG/C.

***2021 AFSN Annual Meeting & Symposium***

The Philippines National Police, the host for this year's virtual AFSN Annual Meeting & Symposium, has made good progress on the preparation for the upcoming meeting, which will be on 14 to 15 October, with pre-event activities on 12 October. The full programme will feature plenary lectures, workgroup/committee scientific sessions, business meetings, as well as the Annual General Meeting. Pre-event activities include retreats and vendors' workshops. I would like to suggest all member institutes to encourage your staff to leverage on this opportunity to learn, share and benefit from the range of speakers. Please refer to the article inside this newsletter for more details.

***Postponement of Board Election***

As stated in our AFSN Constitution, each elected Board Member will serve a term of 2 years. The current Board was elected in 2019 and hence the term will run till 2021. However, due to the ongoing pandemic crisis where many activities have been put on hold and members have not been able to meet physically for an Annual Meeting, a proposal was put forward for the Board election to be postponed by a year, that is, only taking place in 2022. We are happy to announce that this proposal has the support of all member institutes and the Board Election will hence be held at the AGM in 2022.

***Newsletter***

Our AFSN Newsletter ForensicAsia, has been receiving very strong support from all member institutes and the last call for paper has resulted in an overwhelming number of papers received, so much so that we need to split the papers into two issues. The AFSN Board has discussed with the Editorial Board and we will introduce an award "Best Paper of the Year" to be given to the scientific paper that garners the highest score from the Guest Editors and the Board Members. We hope that this award will encourage more quality research papers to be submitted to ForensicAsia. My appreciation goes to the entire editorial team and editor Dr Lui Chi Pang who have done an excellent job in the production of this newsletter.

I hope you will enjoy reading this issue. Keep safe and take care.

***Dr Angeline Yap***
***AFSN President***
***Health Sciences Authority***
***Singapore***

# Editor's Address

Dear colleagues and members of AFSN,

As we are experiencing the second year of the pandemic and still could not see the light at end of the tunnel, I am so excited with our members in the unceasing quest for advancing forensic science in the midst of uncertainty in our communities and the world at large. Indeed, we have received articles in AFSN News about the online meeting in CSI Workgroup as well as the joint webinar in Illicit Drugs Workgroup/ Quality Assurance & Standards Committee in June this year. Most important, our colleagues from the Philippine National Police will be hosting the 13th AFSN Annual Meeting and Symposium this October online.

In this Issue, we have a total of 5 technical articles and 4 case studies, including, crime scene investigation, forensic biology, toxicology, Illicit Drugs, fires and explosions and digital evidence which show the applications of technology and forensic techniques in solving crimes and improving our forensic investigation.

Once again, I would like to thank those who have supported ForensicAsia by contributing your valuable research and studies, our guest editors who spent their precious time in reviewing the articles, and last but not least, our editorial assistants who have helped in the administrative matters and have designed the artwork for the online publication of this new Issue.

Happy reading and continue to stay safe.

***Dr Lui Chi Pang***
***Editor***

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# Editorial Committee



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***For contribution of articles, please read the guidelines at www.asianforensic.net.***

# Content

# 13th Asian Forensic Sciences Network Annual Meeting and Symposium

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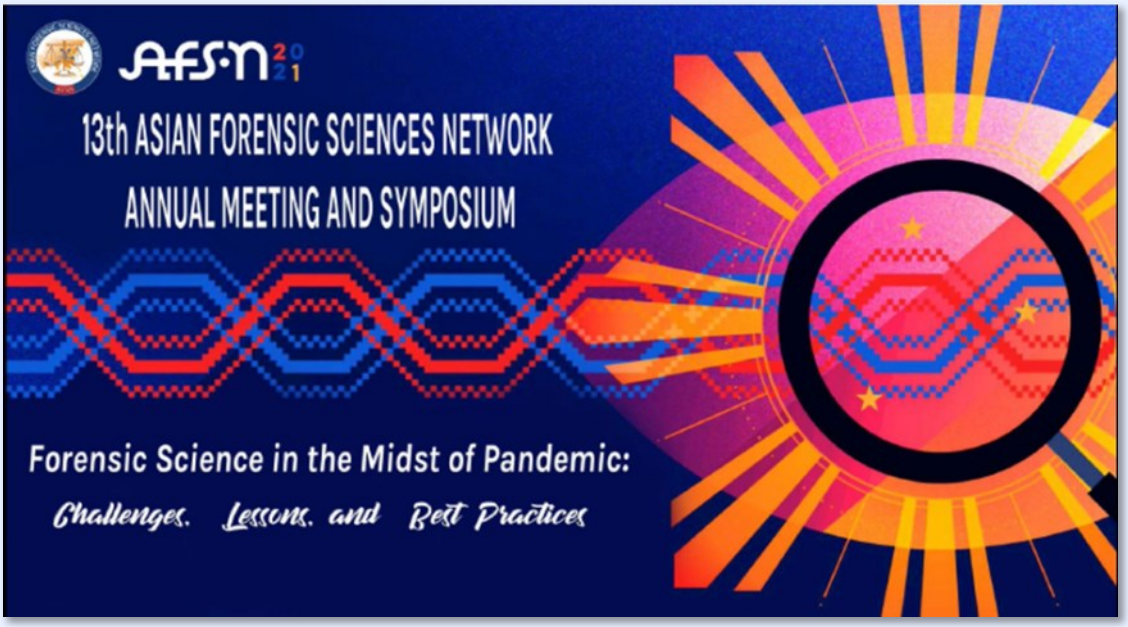


The 13th Asian Forensic Sciences Network Annual Meeting and Symposium will be held in October 14 -15, 2021 (Pre-conference activities to be held in October 12, 2021). The format is virtual this year, which means more members can come together to attend this annual event without the challenges in arranging travel and accommodation needs.

The theme for this year is "Forensic Science in the Midst of Pandemic: Challenges, Lessons, and Best Practices". It is timely for us to share how we tackle our difficulties in the past years and the lessons we learnt.

Registration is now open, and we are calling for submission of abstract. Please go to the meeting's website https://www.afsn2021.com/ for more information.

See you in October!

# Crime Scene Investigation Workgroup Meeting on Firearms/Shooting Reconstruction, 25 June 2021

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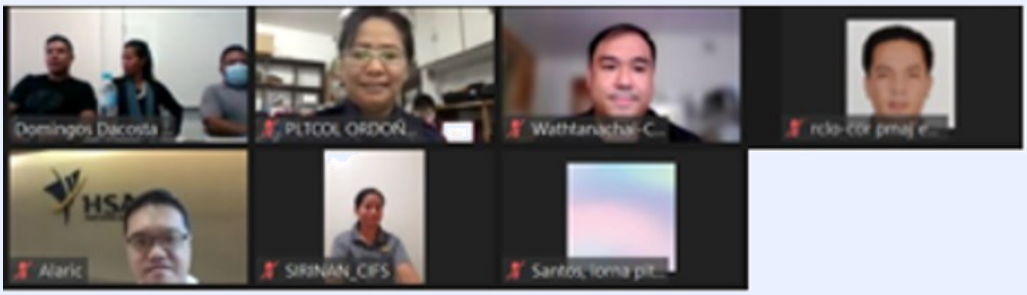
The AFSN Crime Scene Investigation Workgroup (CSIWG) met on 25 June 2021 to share case studies and discuss matters on Firearms and Shooting Reconstruction. More than 30 participants from AFSN member institutes attended and heard from speakers Superintendent Mohd Hazazi bin Othman and Mohd Nizam bin Husain from the Royal Malaysia Police Forensic Laboratory, Police Major Jasmin Nova Kibir-Garcia from the Philippine National Police Crime Laboratory, and Senior Forensic Scientist Liu Jing from the Health Sciences Authority, Singapore. The session was engaging and members took back insights on 3-d scene analysis for testing various propositions, firearms/ballistics examination and laser scanning technologies.

Crime scene investigation involves many related disciplines and the CSIWG looks forward to collaborating with experts from other forensic areas to learn from and advance crime scene work.

# AFSN Joint IDWG/QASC Webinar on Method Validation and ATS Synthesis

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To strengthen our expertise as well as the cross-pollination of ideas through interaction amongst our members, the IDWG and QASC organized a Webinar on 30 June 2021. We invited Ms Cheow Pui Sze, Senior Scientist of the Chemical Metrology Laboratory, Health Sciences Authority, Singapore, to present a workshop in Method Validation. It was a 3-hour talk in which Ms Cheow went into details of the fundamental principles, strategies and steps in validating a new analytical method. This workshop was very well received with over 140 members who registered for this event.


After the first part of the Webinar, the Illicit Drugs Working Group was honoured to have 2 speakers, Dr Paul Kirkbride and Dr Martin Johnston, both from the Flinders University - Adelaide, South Australia. Dr Kirkbride shared on “Current Clandestine Laboratory Research: Routes to Benzaldehyde and Phenyl-2-propanone” and Dr Martin shared on “Novel Synthetic Methods of Amphetamine-Type-Stimulants (ATS) Synthesis”. Both speakers gave an interesting overview on their research work which would be beneficial and insightful to our working group members.

# Simultaneous Identification Of Organic And Inorganic Explosive Particles Collected With Transparent Tapes By Laser Confocal Raman Imaging Technology

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## Abstract

This study aims to develop a tape lifting method to collect trace explosive particles in bombing cases and a non-destructive method to identify explosive particles on the sticky side of transparent tapes directly. Hexogen (RDX) and potassium chlorate ($KClO_3$) were selected to be the target organic and inorganic explosive components respectively. One kind of fingerprint tape commonly used to remove and store fingerprints in China was used to collect explosive particles followed by particle fixation on glass slides. Laser confocal Raman spectroscopy and imaging technology were applied to identify the suspicious particles in the targeted area of the tape directly, without peeling the tape off the glass slide. The Raman band range of 870~900 and $cm^{-1}$ 470~500 $cm^{-1}$ were selected respectively for obtaining component and distribution information of RDX and $KClO_3$.

In bombing cases, an appropriate sampling method is the basis for accurate identification of explosives. Due to the convenience, various fingerprint tapes are commonly used as sampling media for collecting explosives and raw materials from the manufactured place of home-made explosives. Compared with other solvent-based swapping methods, the tape lifting method does not damage fingerprints, DNA and other potential evidence. However, it is not commonly used to collect explosives due to the lack of corresponding identification methods. Raman spectroscopy has been identified as a powerful technique for molecular identification of organic and inorganic explosives, providing molecular fingerprints for many kinds of explosives in a few seconds or minutes. It requires little or no sample preparation, which is extremely useful for non-destructive analysis of explosives on the sticky side of the tape. Furthermore, combined with the advantages of laser confocal Raman spectroscopy with digital imaging, Raman imaging technology provides spatial and spectral information of the different components simultaneously and non-destructively [1, 2].

## Materials and Methods

### *Sample information*

$KClO_3$ was purchased from Tianjin Tianda Chemical Industry Co., Ltd. RDX was purchased from Xi'an modern chemistry research institute. One kind of fingerprint tape (Crystal Lear, China) commonly used to remove and store fingerprints was applied to collect explosive particles.

### *Sample preparation*

RDX and $KClO_3$ were passed through a 100-mesh sieve respectively. 50 μg of RDX and 50 μg of $KClO_3$ powders were weighed, mixed and spread on a clean table in an area of 10×10 $cm^2$. The powders were collected with the sticky side of the fingerprint tape followed by particle fixation on glass slides. Raman imaging analysis was performed to a targeted area of the fingerprint tape with suspect particles to obtain the distribution information of different components, without peeling the tape off the glass slide (Figure 1).

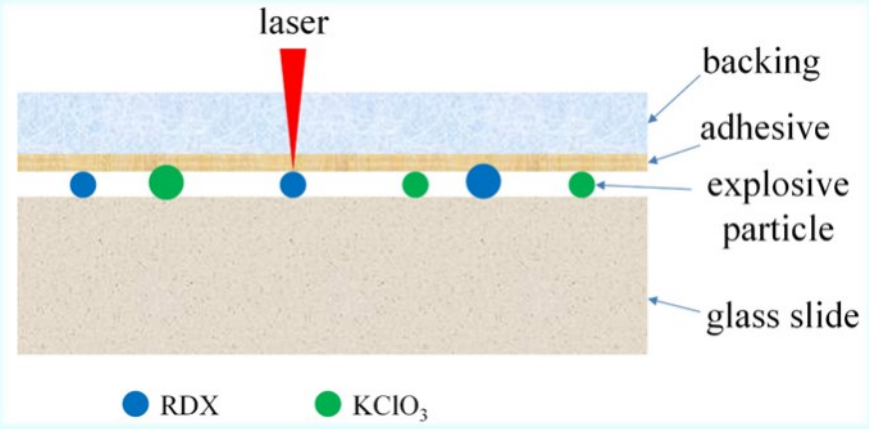


*Figure 1: The diagram of identification of explosive particles on the adhesive side of the transparent tapes by laser confocal Raman microscopy.*

***Raman imaging analysis***

Raman spectra of samples were obtained with a laser confocal Raman spectroscopy of LabRAM HR Evaluation (Horiba Jobin Yvon, Paris, France) controlled by the LabSpec6 software. The measurement conditions of Raman imaging were listed in Table 1. Under the microscope of Raman spectrometer, one of the suspicious particles would be selected as the origin to facilitate the Raman focusing. The Raman images were recorded with a ×50-LWD objective (NA=0.5, Olympus, Tokyo, Japan). By extracting individual features such as Raman peak position, peak range, or peak intensity from the recorded 2D arrays of spectra, different components particles on the tapes could be marked in different colors in one image, making the results concise and intuitive.

| Measurement parameters | Parameter settings |
|---|---|
| Excitation wavelength | 473 nm (Solid-state laser) |
| Spectrum range | 100~1700 $cm^{-1}$ |
| Incident laser power | 13.3 mw |
| Confocal pinhole size | 100 μm |
| Acquisition spectral resolution | 0.7 $cm^{-1}$ |
| Acquisition time | 500 ms |
| Accumulation times | 1 |
| Imaging area | 1 mm × 1 mm |
| Imaging steps | 80 μm |
| Imaging time | 520 min |

*Table 1 Measurement conditions of Raman imaging*

***Results and Discussion***

Raman spectral libraries were used for identifying the components of all samples by spectral comparison. The spectra of RDX and $KClO_3$ were shown in Figure 2. The characteristic Raman bands of RDX and $KClO_3$ were listed in Table 2. The principle of confocal Raman spectroscopy facilitated the identification of explosive particles on the sticky side of the tape.

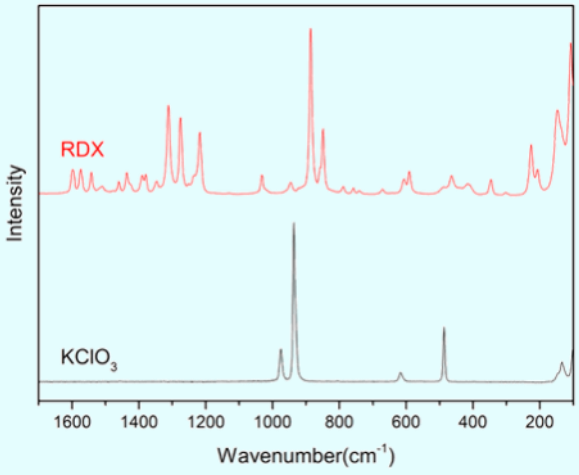


*Figure 2: Raman spectra of RDX (above) and $KClO_3$ (below) particle*

| Explosive or component | Formula | Raman shift ($cm^{-1}$) | Frequencies assignments to molecular vibrations |
|---|---|---|---|
| RDX | $C_3H_6N_6O_6$ | 1591 | $NO_2$ asym str |
| | | 1432 | H-C-N bend |
| | | 1307 | $CH_2$ wag, $CH_2$ twist and N-N str |
| | | 1270 | $CH_2$ scis or N-N str |
| | | 1213 | $CH_2$ rock |
| | | 941 | C-N str, $CH_2$ rock and N-N str |
| | | 881 | C-N str and N-N str |
| | | 844 | N-N str and $NO_2$ scis |
| | | 602 | O-C-O str |
| | | 460 | ip ring bend |
| Potassium chlorate | $KClO_3$ | 978 | $ClO_3^-$ asym str |
| | | 940 | $ClO_3^-$ sym str |
| | | 620 | $ClO_3^-$ sym def |
| | | 488 | $ClO_3^-$ asym def |

*Table 2. Summary of the main Raman bands from the spectra displayed in Figure 2 and their assignment with the fundamental vibrations. (asym: asymmetric, bend: bending, def: deformation, ip: in-plane, oop: out-of-plane, rock: rocking, scis: scissoring, str: stretching, sym: symmetric, twist: twisting, wag: wagging)[3].*

During imaging analysis, thousands of spectra were obtained. Based on the difference between the Raman characteristic bands of RDX and $KClO_3$, the intense Raman band at 881 $cm^{-1}$ of RDX and medium band at 488 $cm^{-1}$ of $KClO_3$ can be used as the characteristic peaks to obtain distribution information. Correspondingly, the band range of 870~900 $cm^{-1}$ and 470~500 $cm^{-1}$ were selected respectively for obtaining imaging distribution of RDX and $KClO_3$ (Figure 3(a)~3(c)). From the spectrum information collected during the imaging process (Figure 3(d)~3(e)), there was no interference from the backing and adhesive of the tape to identify explosives.

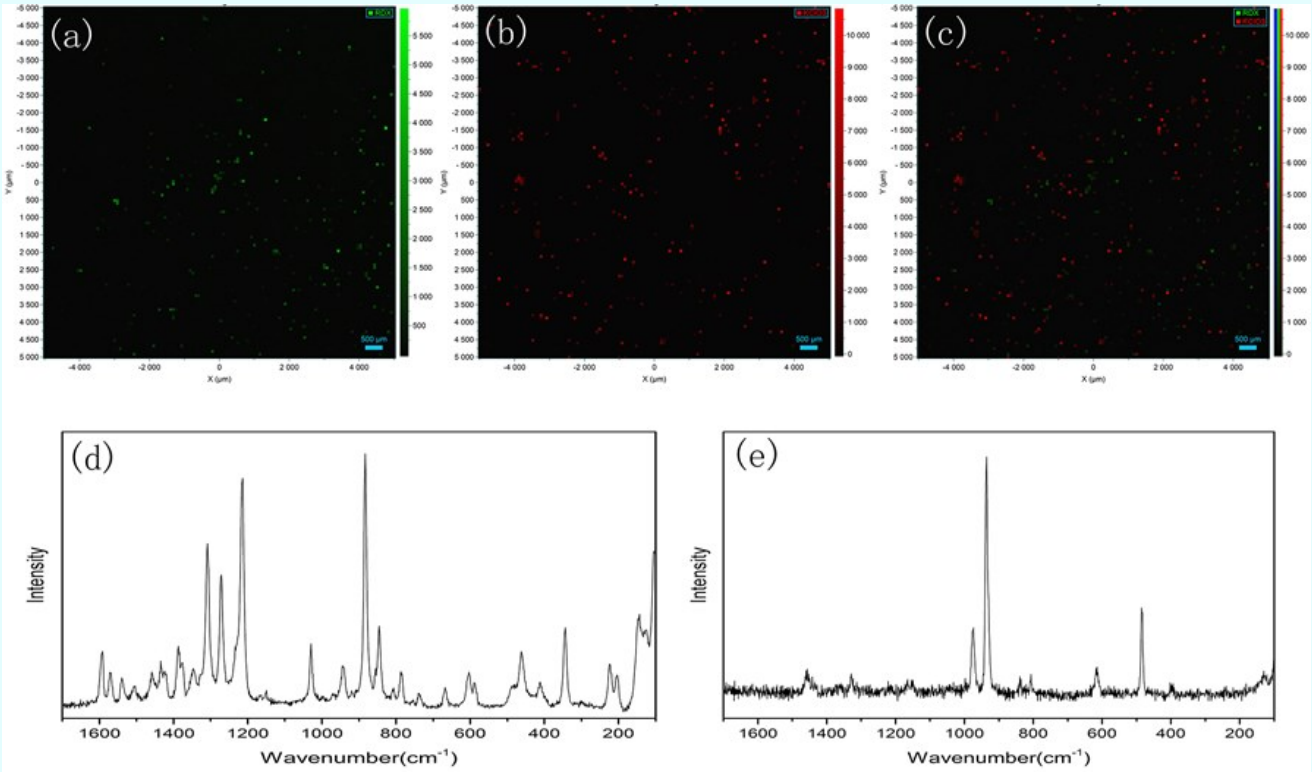


*Figure 3: The distribution of explosive particles by selecting the characteristic Raman bands. (a) RDX distribution (870~900 $cm^{-1}$); (b) $KClO_3$ distribution (470~500 $cm^{-1}$); (c) RDX (green) and $KClO_3$ (red) distribution; (d) and (e) is the Raman spectra of RDX and $KClO_3$ particles on the sticky side of the tape respectively. (Spectral acquisition time: 500 ms; acquisition times: 1; laser power: 13.5 mW).*

Compared to the solvent-based swabbing method, tape lifting is an ideal method to collect explosives and raw materials at crime scenes. Raman imaging technology is suitable for obtaining the distribution and component information of explosive particles on the tape. The transparent tape can restrict the particles to the same focal plane, which makes the sample more suitable for Raman imaging analysis. The combination of Raman imaging technology and the tape lifting method shows great potential for identifying forensic samples by providing chemical and spatial information.

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# Rapid Screening of Amphetamine, Methamphetamine and Methylenedioxymethamphetamine in Urine by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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## Abstract

A simple and rapid screening method for the determination of amphetamine (AMP), methamphetamine (MA) and methylenedioxymethamphetamine (MDMA) by liquid chromatography tandem mass spectrometry (LC-MS/MS) has been developed and validated. Acidified methanol was used to extract drugs from the urine sample. After vortex and centrifugation, the supernatant was allowed to pass through a dual mode extraction (DME) column before LC-MS/MS analysis. The limit of detections (LODs) of AMP, MA and MDMA were as low as 3 ng/mL.

## Introduction

Amphetamine (AMP) and methamphetamine (MA) are sympathomimetic phenethylamine derivatives with prominent central stimulant activity. AMP and MA have been widely used clinically for the treatment of attention deficit hyperactive disorder (ADHD), narcolepsy and obesity. Due to its low cost and wide availability, MA is one of the most abused drugs worldwide. According to the latest published figures by the Action Committee Against Narcotics (ACAN) of the HKSAR, MA continued to be the most common type of psychoactive substance abused, with 827 reported abusers in the first half of 2019 [1]. Methylenedioxymethamphetamine (MDMA) is structurally related to AMP and MA (Figure 1). In low dosages, it can cause feelings of pleasure; whereas in high dosages, it can cause hallucination. Since these drugs are commonly abused, it is imperative for the laboratory to develop a protocol to determine their presence. In this report, the authors describe a simple and rapid screening method to detect the presence of AMP, MA and MDMA in urine. The urine samples were extracted with acidified methanol. The extracts, after vortex and centrifugation, were passed through the dual mode extraction (DME) columns before LC-MS/MS analysis. The DME column is specially highlighted in this work as a novel method to clean up urine samples.

## Materials and Methods

Certified reference materials of AMP, MA and MDMA as well as their deuterated analogues of MA-d5 and MDMA-d5 were purchased from Lipomed (Arlesheim, Switzerland) and Cerilliant (Round Rock, TX, USA). Dual mode extraction (DME) columns were purchased from Biotage (Charlotte, NC, USA). HPLC grades of methanol and acetonitrile and analytical grade of formic acid (FA) were acquired commercially and used as received. The urine samples used in this validation study were authentic ante- and post-mortem samples submitted to our laboratory for toxicology analysis. These samples were screened to ensure no interferences were observed at the retention time of the abuse drugs of interest.

### Procedure

A 0.2-mL urine sample which was spiked with MA-d5 and MDMA-d5 as internal standards (ISTDs) was diluted with 0.4 mL of 1% FA in methanol as a crash solvent for the extraction of drugs from the urine. The sample was vortex mixed, followed by centrifugation at 13000 rpm. The supernatant was cleaned up by passing through a DME column. Urine eluants from the column were directly analyzed without any reconstitution using an Agilent 1260 LC system (Agilent Technologies, Wilmington, DE, USA) coupled to a SCIEX 5500 QTRAP mass spectrometer (Applied Biosystems, Foster City, CA, USA) for positive mode electrospray (ESI+) analysis. Chromatographic separation was achieved by an Alltima C18 column (2.1 ´ 150 mm, 5 mm) using 0.1% formic acid (A) and acetonitrile (B) as the mobile phases. Gradient elution of mobile phases A and B at a flow rate of 0.2 mL/min was adjusted. The gradient started at 2% B, then increased to 10% B at 5 mins and gradually climbed to 40% B at 20 mins. The concentration of B finally attained 80% at 22 mins and was held for 3 mins before returning to the initial condition of 2% B at 26 mins. The total run time was 35 mins. Quantitative analyses of AMP, MA and MDMA were performed in the multiple reaction monitoring (MRM) with optimized parameters for declustering potential (DP), entrance potential (EP), collision energy (CE) and cell exit potential (CEP) (Table 1).

## Method Validation

Method validation was performed in accordance to the American Academy of Forensic Sciences (AAFS) [2]. Performance parameters of interference study, limit of detection (LOD) and matrix effect were evaluated.

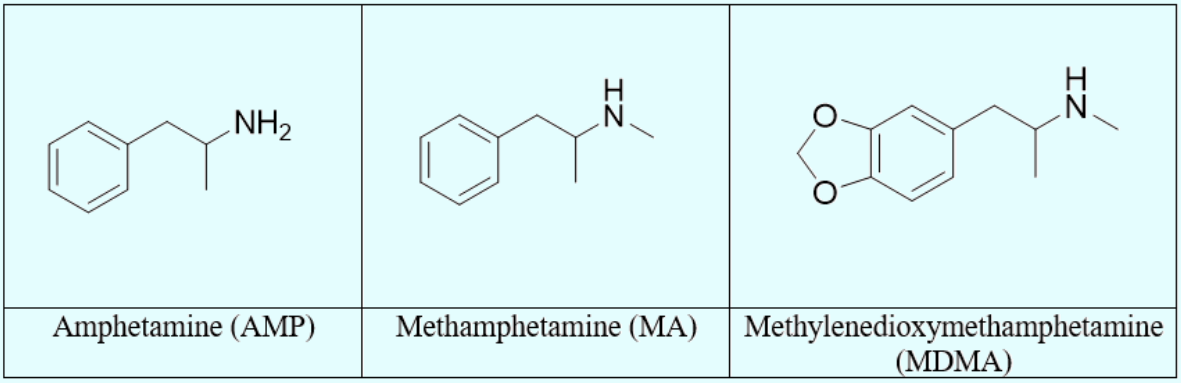
### *Interference Study*

Interference was studied by analysis of the urine samples submitted to our laboratory for toxicology analysis. These samples covered a wide spectrum of drugs such as amphetamine-type, opiates, benzodiazepines, antihistamines, analgesics, Z-drugs, antibiotics and antipsychotics. None of these analytes gave any responses at the retention time of AMP, MA and MDMA.

*Table 1: Diagnostic ions and the optimized MS conditions for AMP, MA and MDMA*

| Analyte | Diagnostic ions | | | Setting | | | |
|---|---|---|---|---|---|---|---|
| | RT (min) | Precursor ion (*m/z*) | Product ion (*m/z*) | DP | EP | CE | CXP |
| AMP | 6.50 | 136.2 | 90.9 | 71 | 10 | 25 | 17 |
| | | | 119.1 | | | 11 | 10.5 |
| MA | 7.44 | 150.3 | 91.0 | 66 | 12 | 22 | 7 |
| | | | 119.1 | | | 14 | 9 |
| MDMA | 8.35 | 194.2 | 163.2 | 73 | 10 | 15 | 9 |
| | | | 105.2 | | | 30 | 8 |
| MA-$d_5$ | 7.39 | 155.3 | 92.2 | 66 | 12 | 22 | 7 |
| MDMA-$d_5$ | 8.29 | 199.2 | 165.1 | 73 | 10 | 15 | 9 |

Note: RT: Retention time. DP: Declustering potential. EP: Entrance potential. CE: Collision energy. CEP: Cell exit potential. The first and second ions denote the quantitative and qualitative transition product ions, respectively.




| Amphetamine (AMP) | Methamphetamine (MA) | Methylenedioxymethamphetamine (MDMA) |
|---|---|---|

*Figure 1. Molecular structures of amphetamine (AMP), methamphetamine (MA) and methylenedioxymethamphetamine (MDMA)*

***Limit of Detection (LOD)***

The United Nations Office on Drugs and Crime (UNODC)[3] and the Society of Forensic Toxicologists (SOFT)[4] independently published guidelines for the forensic analysis of drugs facilitating sexual assault (DFSA) and other criminal acts. In these guidelines, they have defined the minimum required performance levels (MRPLs) which are technical performance parameters that laboratories should comply with when testing for the presence of DFSA related substances. UNODC requests laboratories to be capable of detecting AMP, MA and MDMA at as low as 10 ng/mL, whereas SOFT sets the detection limit for the same drugs at 50 ng/mL. The LODs of these drugs in this method were determined by replicate analyses (n=7) of the urine samples fortified at the lowest non-zero calibrator.

## Result and Discussion

In this work, we employed a DME column to clean up the urine samples. Unlike solid phase extraction columns, DME columns do not require any pre-conditioning step, nor is there any washing of columns after sample loading. The eluant was collected directly in a vial for instrumental analysis.

No exogenous interference was observed at the retention times of the AMP, MA and MDMA on LC-MS/MS analysis of the seven ante- and post-mortem urine samples. In addition, two urine samples which were fortified with ISTDs only were analyzed. No interference was observed, showing that the stable isotope-labeled ISTDs were pure. The LODs of AMP, MA and MDMA were determined by analyzing the chromatographic spectrums of fortified urine samples (n=7) at a low concentration (i.e. 3 ng/mL). The precisions which were expressed as coefficient of variation (CV), of AMP, MA and MDMA were 10%, 6% and 7%, respectively. In all the seven replicate analyses, the ion ratios (Qualifier transition/Quantifier transition) and relative retention time (RRT) were within ±20% and ±2.5% of those in the QC sample which was prepared by a blank urine sample fortified with the analytes at 15 ng/mL. All these ions showed satisfactory signal-to-noise ratios (i.e. S/N ≥ 3) (Figure 2).

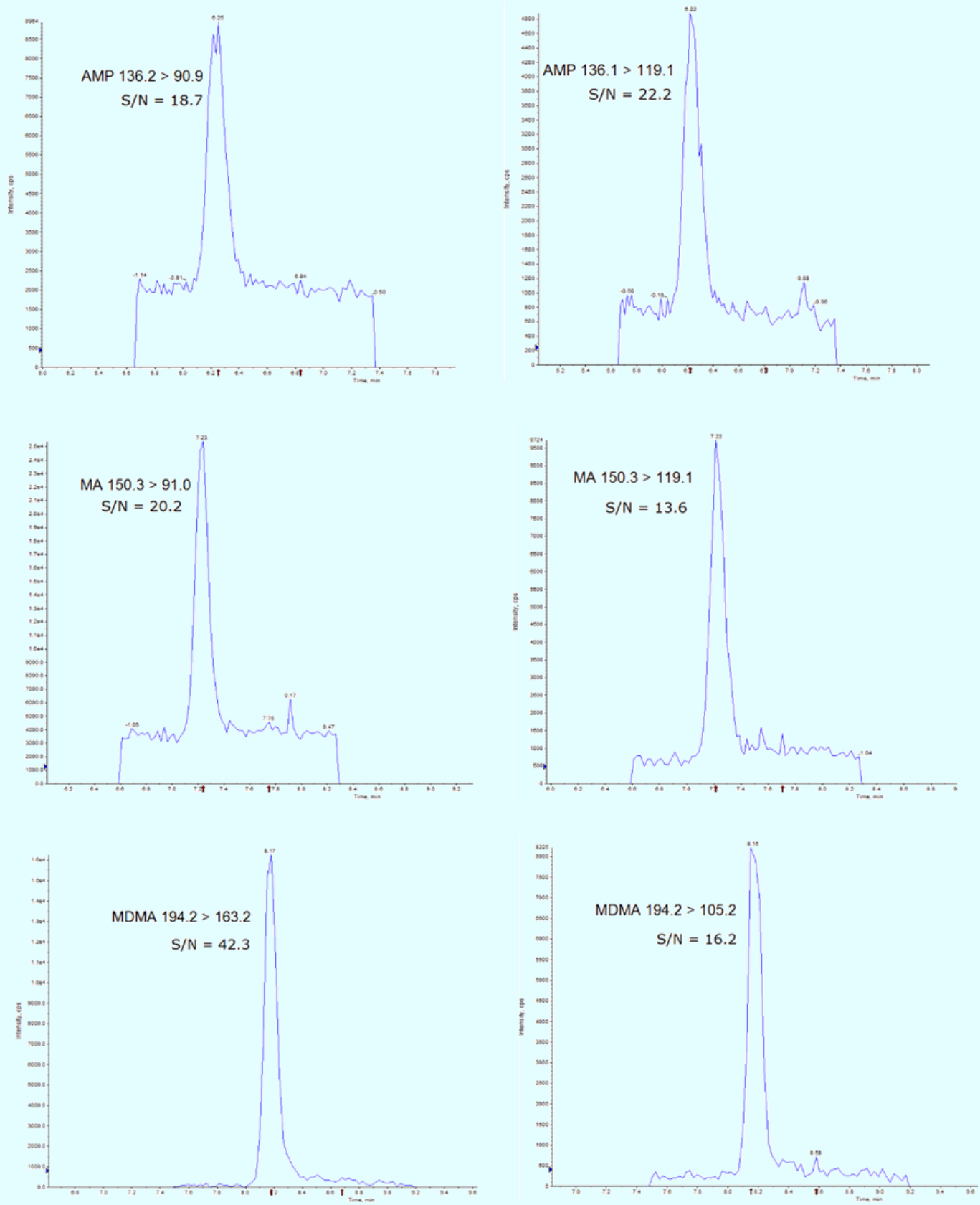



*Figure 2. Chromatograms of AMP, MA and MDMA at 3 ng/mL*

## Conclusion

A rapid LC-MS/MS screening method using DME columns for cleaning up the urine samples for the detection of AMP, MA and MDMA was developed and validated. This method has the advantage of consuming only a small volume of urine sample (i.e. 0.2 mL). Besides, the sample preparation is fast as it does not require liquid-liquid extraction which is labor intensive. The LODs were determined as 3 ng/mL which are lower than the values listed in UNODC and SOFT. This screening method is deemed appropriate for the forensic analysis of DFSA and other criminal acts. Owing to the versatility of the current method, expansion of the scope to include more abused drugs is underway.

## Acknowledgment

The authors would like to express their sincere thanks to Dr. KY To for his comments and support in the present work. Special thanks are indebted to Dr. WM Sin, the Government Chemist, and Mr. BKK Cheung, the Assistant Government Chemist for their support and encouragement. Lastly, technical supports from Ms. WL Lee and Mr. WY Wong are acknowledged.

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# Beijing Intelligent Scene Investigation Technology

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## Abstract

Beijing Forensic Science Institute has developed a new investigation mode which simultaneously process the scene investigation, evidence collection, information entry, information comparison, case serialization and information feedback. A series of efficiency increasing devices have been developed to support this system by enhancing the investigators' ability to quickly and easily handle the scene and collect evidence.

## 1. Foreword

Intelligent Scene Investigation（ISI）provides six-stage synchronization. When the crime scene is investigated, all six procedures (the scene investigation, evidence collection, information entry, information comparison, case serialization and information feedback) are simultaneously processed. In the past, investigators were only responsible for scene investigation and evidence collection. Information entry, information comparison, case serialization and information feedback were completed when they returned to the office. Under ISI, all the work is completed at crime scene. Thus, six-stage synchronization has been partly realized. There are three core supporting technologies: intelligent guide, unidirectional IOT and synchronization of information collection (comparison and feedback). We have also developed a series of ISI devices centered around ISI terminal which connects to our mobile public security network and handles data transmission. Other auxiliary devices play a role in evidence collection and uploading data to ISI terminal.

## 2. Intelligent Guide Technology

We have digitized the standard of procedure (SOP) and placed it into the ISI terminal, making years of experience and practical work more accessible and aiding our ultimate aim of standardizing scene investigation. The investigators at a crime scene follow the guide in the ISI terminal to carry out their investigation, collection and entry work. When the scene investigation is finished, the information entry will be completed simultaneously. In order to achieve this goal, we have made scene investigation procedure topologies (see Figure 1) covering different kinds of cases. With the help of programming technique, we have digitized these topologies to ISI terminal and finally formed this operation interface into an intelligent guide (see Figure 2).

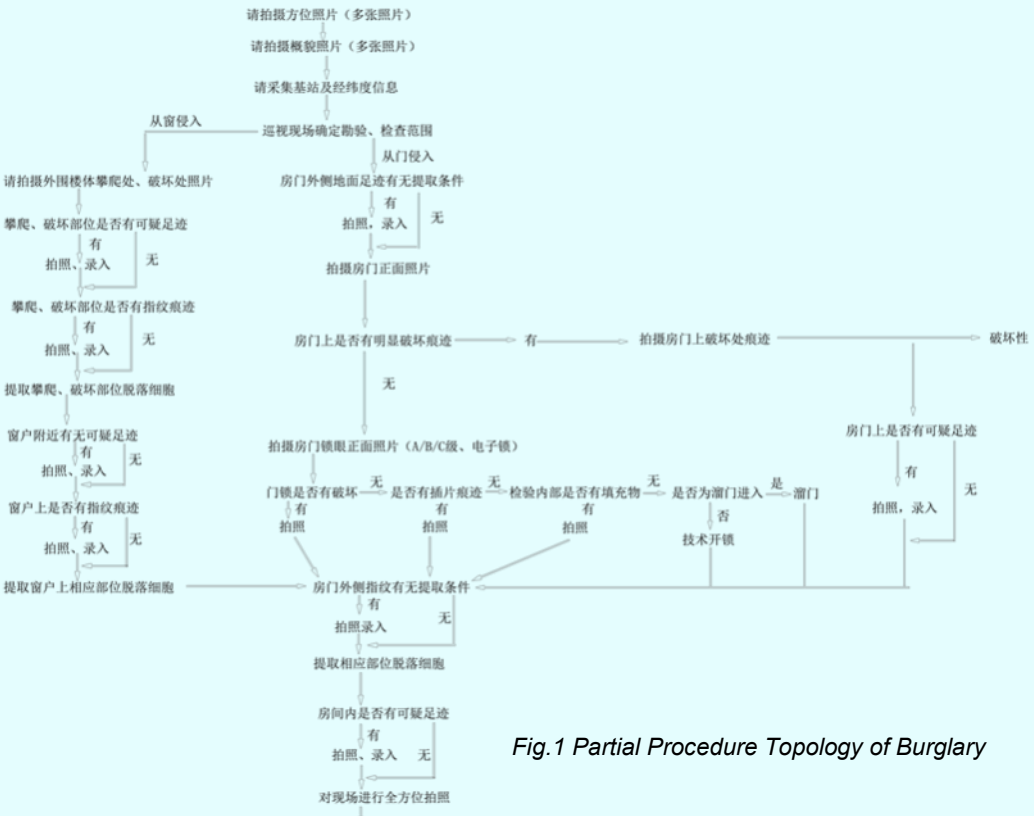


*Fig.1 Partial Procedure Topology of Burglary*


*Fig.2 Operation Interface of Burglary Intelligent Guide*

As you can imagine, whether you are a veteran or novice in scene work, it will lead you to the same working process by using the terminal. The information we collected and entered at crime scene will also be same. However, what needs to be illustrated is that the guide is alterable and open. Our work procedure is changing with society. Refreshing the topologies will ensure the guide advances with the times.

## 3. Unidirectional IOT Technology

At present, there are many dedicated evidence collection devices, but most of them are standalone. They cannot transmit the evidence to the database directly for comparison and case serialization. We have developed these standalone devices so they can upload their data to ISI terminal and then from the terminal to database. However, these developed devices require much attention since they only can upload data to terminal but cannot check or download any data from terminal. This is “unidirectional IOT technology” which is essential for the safety of information. The technology lets the ISI terminal have no trouble connecting with dedicated devices, makes ISI become an open technical system which centered around ISI terminal, and combines with different kinds of dedicated devices. Thus far, we have connected DSLR, ultra-wide spectral fingerprint developing device (UWSF), and a shoeprint scanner to ISI terminal under unidirectional IOT technology. The data collected by these devices can be uploaded to the ISI terminal at the crime scene immediately (see Figure 3-5).

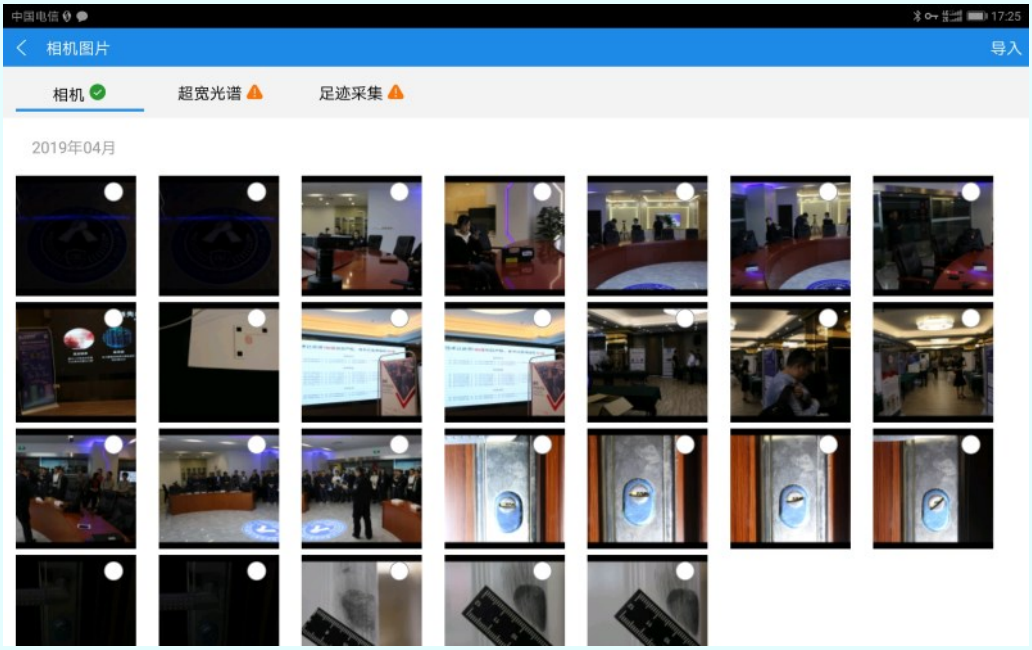


*Fig.3 ISI Terminal visiting photos in DSLR*

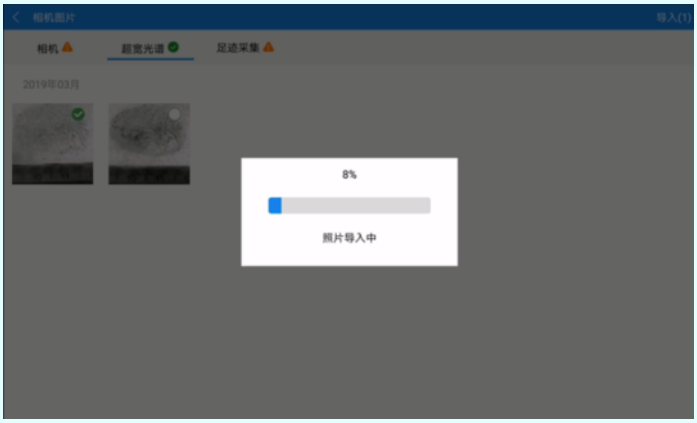


*Fig.4 Fingerprint in UWSF uploading to ISI terminal*


*Fig.5 Shoeprint in Scanner uploading to ISI terminal*

## 4. Synchronization of Information Collection, Comparison and Feedback

The institution has achieved the synchronization of information collection, comparison and feedback in fingerprint and shoeprint field, The details are as follows:

### 4.1 Synchronization of Information Collection, Comparison and Feedback

With the help of unidirectional IOT technology, we have linked ALS fingerprint collector, DSLR and ultra-wide spectral fingerprint developing device to the ISI terminal. Investigator can complete fingerprint collection in most cases and send it to the terminal. The victim's fingerprint collection will be finished by a mobile live fingerprint collector (See Figure 6-9). We have embedded fingerprint excluding program to the ISI terminal, which can exclude the victim's unrelated fingerprint from the print we collected at scene. The whole process takes no more than dozens of seconds (See Figure 10). If the scene fingerprint matches the victim's print, a red star will appear on the top left corner of the matched victim's print in the terminal's excluding interface. Otherwise there will be no mark. For the fingerprints not matched, we will use the ISI terminal to send them to our fingerprint database. The result can be fed back to the terminal in minutes (See Figure 11).

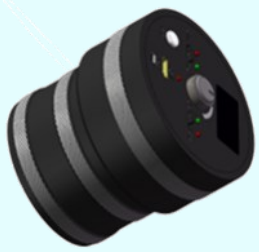

*Fig.6 ALS Fingerprint Collector*


*Fig.7 DSLR*

*Fig.8 Ultra Wide Spectral Fingerprint Developing device*

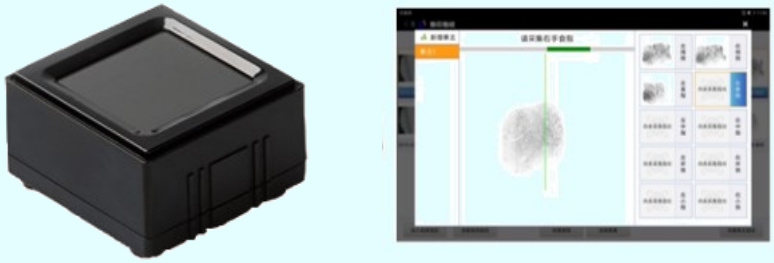

*Fig.9 Mobile Live Fingerprint Collector and the related program*

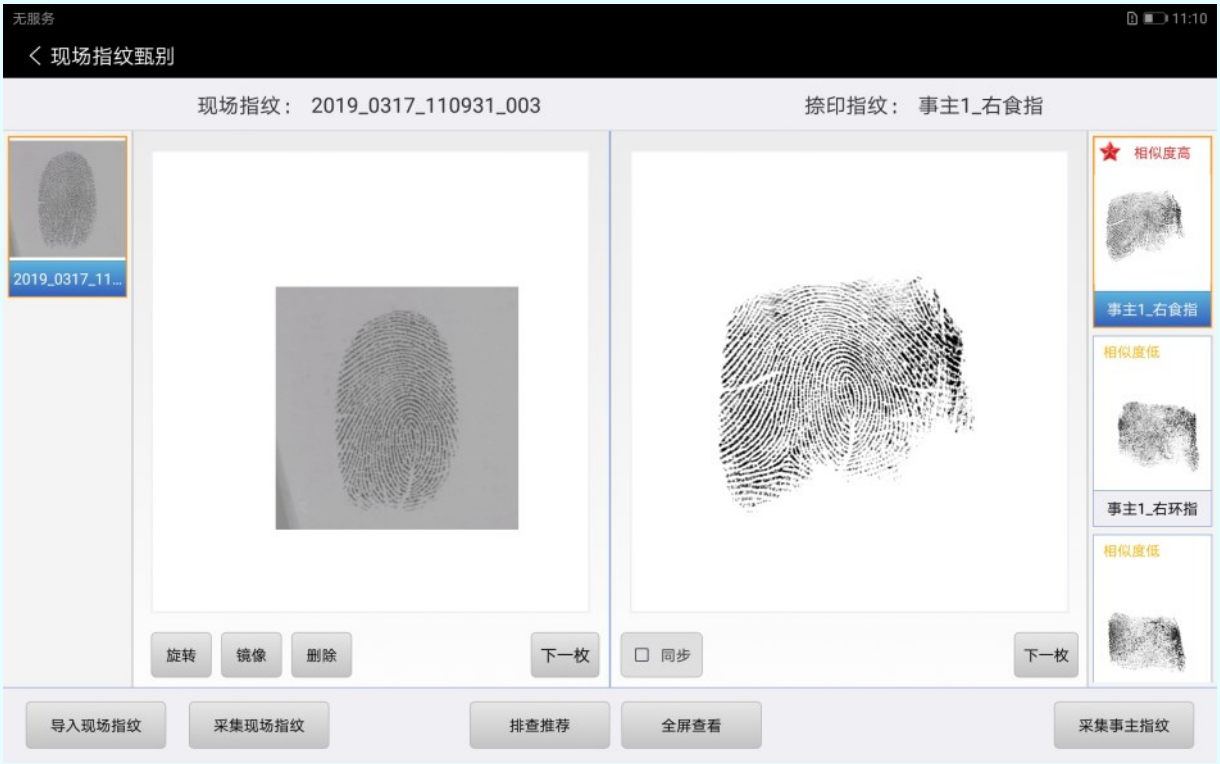


*Fig.10 Fingerprint Excluding Program in ISI Terminal*


*Fig.11 Fingerprint Feedback Interface*

### 4.2 Synchronization of Shoeprint Collection, Comparison and Feedback

The shoeprint collection devices linking to the ISI terminal include the DSLR and mobile shoeprint scanner (See Figure 12). Investigators can handle shoeprint collection work in most cases and transmit the data to ISI terminal. By far, the terminal can upload the shoeprint to the shoeprint analysis system and national shoe type database by the public security network in order to investigate shoe type and serialize cases at the scene (See Figure 13,14).


*Fig.12 Mobile Shoeprint Scanner*

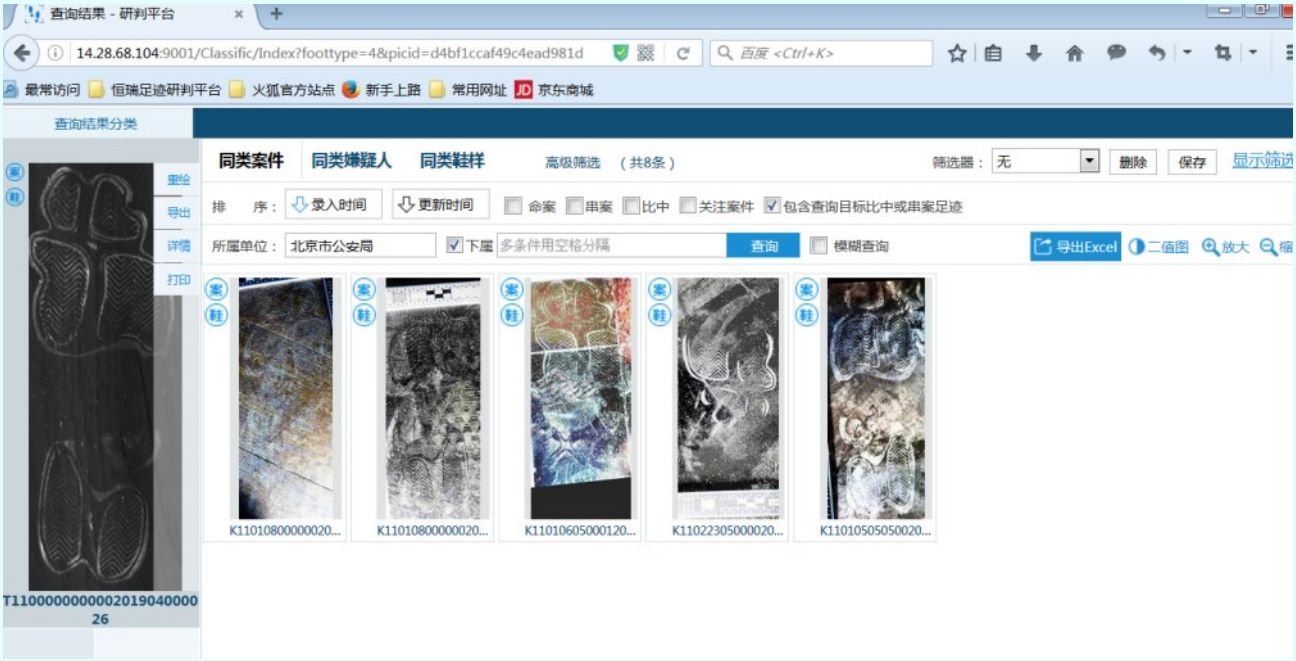


*Fig.13 Uploading data to shoeprint analysis system to serialize cases*

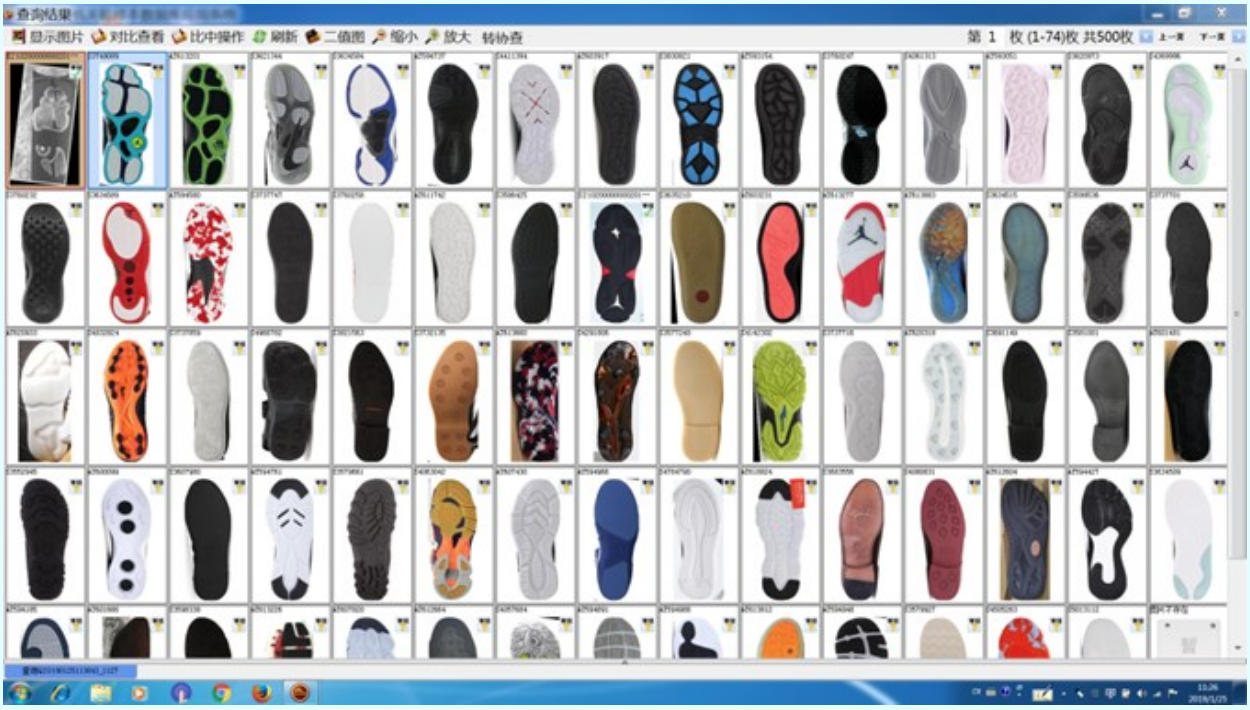


*Fig.14 Uploading data to national shoe type database*

# Identification Of Residues Of Kerosene In Fire Debris In The Presence Of Polyethylene Interfering Compounds

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## Abstract

The aim of this study was to identify kerosene residues in fire debris in the presence of interfering compounds from polyethylene (PE) based household products. Interpretation of chromatograms is difficult in kerosene accelerated fires. If the samples contain PE based products, they produce similar compounds as well as a similar shape/pattern in the chromatogram of kerosene. PE pyrolysis and burnt products appear as triplets or quadruplets and produce a Gaussian pattern in the chromatogram. Therefore; these interfering compounds cause false negative and false positive results. To overcome this misinterpretation of results, the significant consecutive C3 aromatics (castle group) and target compounds of kerosene (C9-C16 alkanes) can be considered since kerosene in Sri Lanka contains aromatics in addition to alkanes relatively with a low abundance when it comes from the refinery.

## Introduction

Fires may be deliberate or accidental. In the case of arson, samples of fire debris are analyzed for residues of ignitable liquids that are used as accelerants. PE is a simple synthetic polymer commonly found everywhere, mainly in packaging. Pyrolysis of polyethylene leads to the formation of a series of “triplets” for each carbon number corresponding to the alkadiene, the alkene and the alkane sometimes forming “quadruplets” [1]. These triplets/quadruplets produce a similar shape/pattern to the kerosene chromatogram. This becomes more difficult as fire debris samples have trace levels of accelerants. Therefore; when interpreting the chromatograms, significant consecutive C3 aromatics (castle group) must also be considered to identify residues of kerosene and avoid false results.

## Materials and Methods

### Chemicals and Instrumentation

Samples of PE (shopping bags), acetone (99%), HPLC grade (Sisco Research Laboratories Pvt. Ltd), unlined cans (Mikro Industries, Sri Lanka), activated charcoal strips (20 mm x 8 mm x 1 mm, Arrowhead Forensics, USA).

Forced convection oven (DK-600DT, Yihder Technologies Co., Ltd, Taiwan), GC/MS (Agilent Technologies 7890B GC, 5977A MSD, 7693 Auto sampler).

### Method

PE samples (10.0 g) were placed in metal cans and labelled appropriately. Controls used were two unburnt neat samples, two samples pyrolysed using the modified destructive distillation method [2] and two samples burnt without kerosene. The remaining were burnt after being spiked with known amounts of kerosene at 5, 10, 15,20,25,50,100,150 and 200 µl. All the samples were then placed in the oven at 80°C for 8 hours for passive headspace extraction under the ASTM E1412 standard practice [3] and finally analysed using the GC/MS. Data interpretation was done using diagnostic pattern recognition and extracted ion profiling where appropriate. NIST library was used for target compound identifications according to the ASTM E1618 guidelines [4].

### GC/MS Settings

| | |
|---|---|
| Initial temperature | 40 $^0$C |
| Initial hold time | 2 min |
| 1$^{st}$ Ramp rate | 5 $^0$C / min up to 90 $^0$C |
| 2$^{nd}$ Ramp rate | 14 $^0$C / min up to 250 $^0$C |
| Hold time | 10 min |
| Final temperature | 250 $^0$C |
| Total run time | 33.5 min |

*Table 1: GC temperature program*

## Results and Discussion

Figure 1 (A) is the Total Ion Chromatogram (TIC) for 0.1% kerosene reference. (B) shows the TIC for the unburnt neat PE sample which contained some alkanes and it might be mistaken for positive responses. (C) and (D) depict the TICs for the burnt and pyrolysed samples which show the specific interfering peaks relevant to PE. These interfering peaks cause masking alkane peaks and also might be misinterpreted as kerosene. Further, (B), (C) and (D) do not contain aromatic peaks relevant to kerosene.

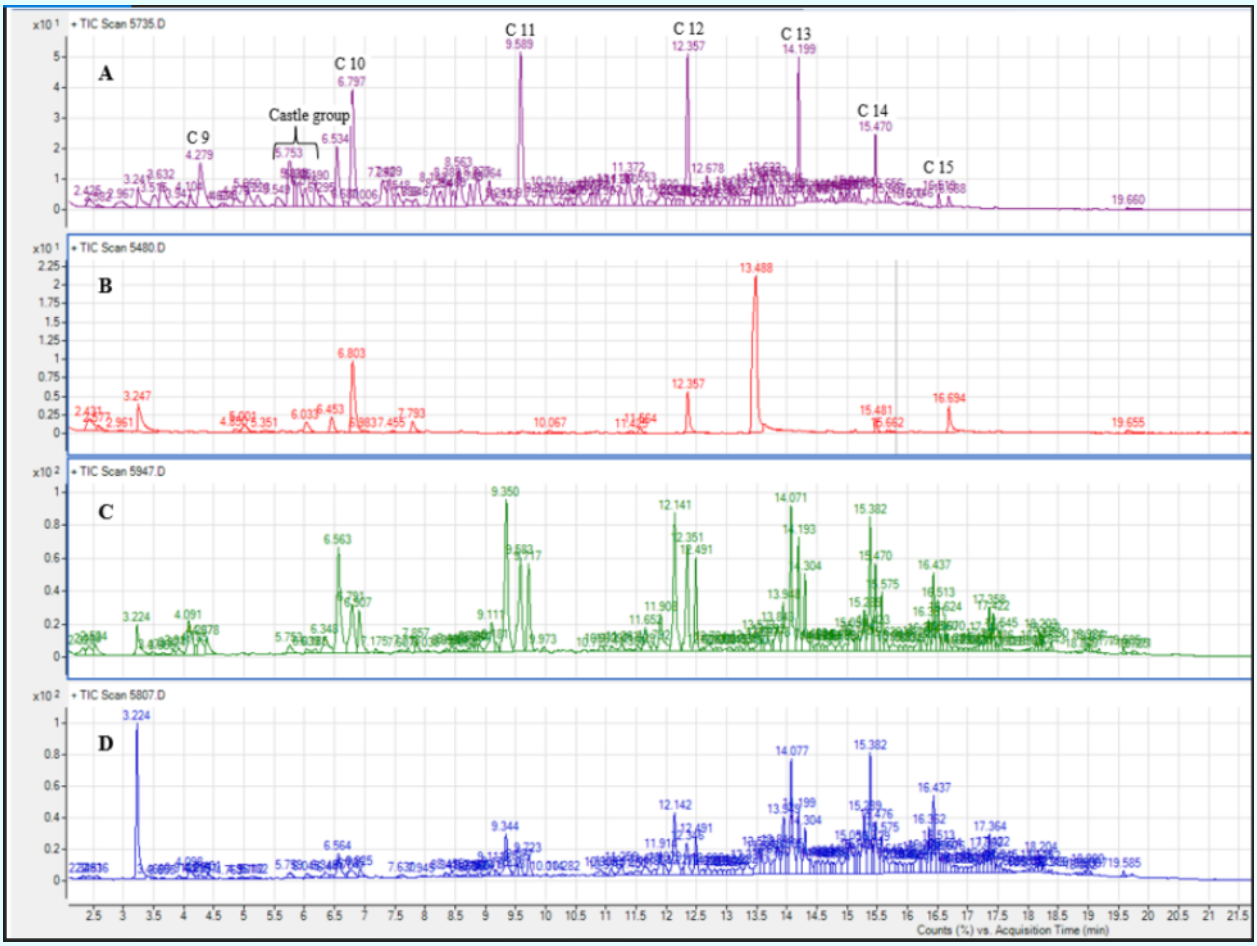


*Figure 1: TICs for (A) 0.1% Kerosene reference, (B) Neat PE sample, (C) Burnt PE sample without kerosene and (D) Pyrolysed PE sample*

Figure 2 depicts the chromatograms of the spiked samples that were selected for the comparisons between the 5, 10, 15, 20, and 200 µl samples. The chromatogram for the 200 µl kerosene spiked burnt sample showed no quadruplets and the alkanes and castle group can be seen clearly which means kerosene is present with no mistake. Further on decreasing the volume of kerosene spiked in the samples showed that the castle group and alkanes remained the same as in the reference kerosene without any pattern distortion although the abundance was decreasing and interfering peaks increasing. The chromatogram for the 20 µl spiked sample showed the margin as the last volume to have prominent alkane peaks and the castle group slightly visible with a Gaussian shape/pattern. The chromatograms for 15, 10 and 5 µl showed a Gaussian shape/pattern, but the alkanes are lower than the alkene and aldehyde peaks and the castle group cannot be seen clearly as it is at a very low abundance in all three chromatograms which means false negative.

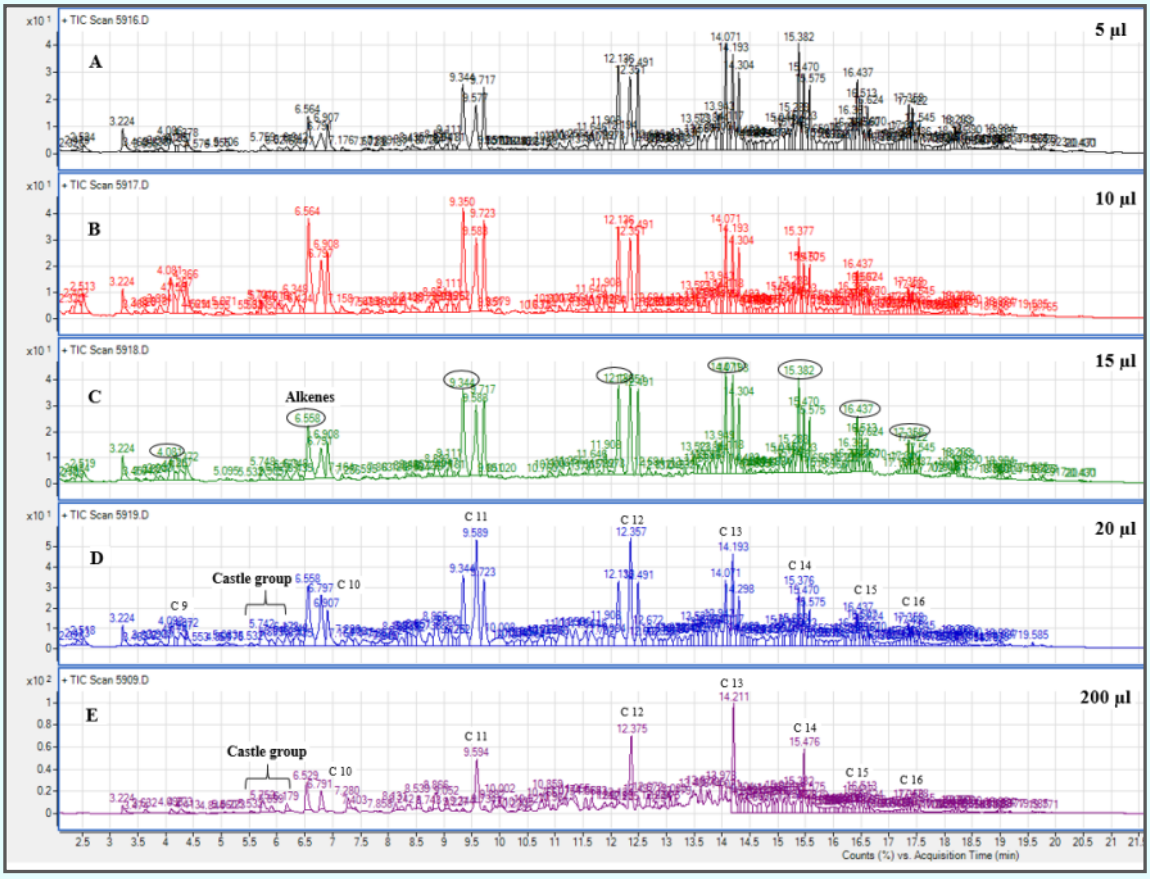


*Figure 2: TICs for burnt PE samples spiked with kerosene of volume (A) 5 µl, (B) 10 µl, (C) 15 µl, (D) 20 µl and (E) 200 µl*

Along with looking at the castle group and target compounds, Extracted Ion Profiles (EIP) of 15 µl and 20 µl spiked samples also helped in confirming the masked accelerants used as they extract the major ions of each compound as shown in Figures 3 and 4. When comparing the two EIPs, the castle group can be seen clearly in both in addition to their prominent alkane profile. Therefore; below 20 µl spiked samples, it is difficult to interpret as positive for kerosene only looking alkane profile in TIC.

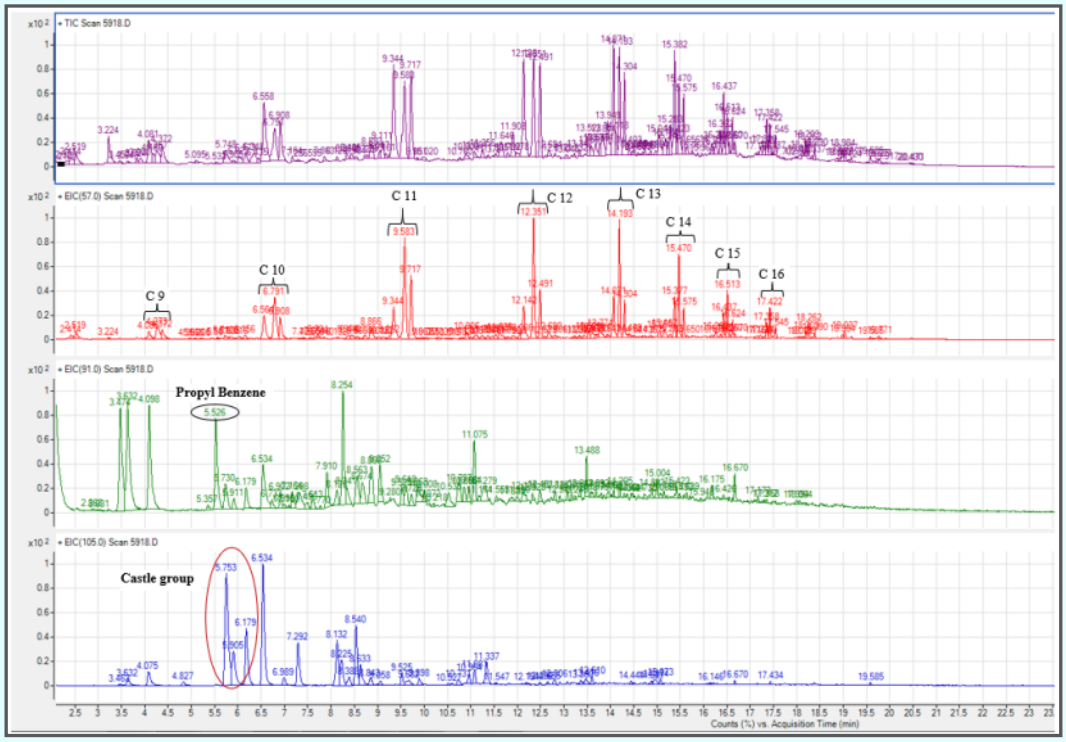


*Figure 3: EIP for sample spiked with 15 µl kerosene*

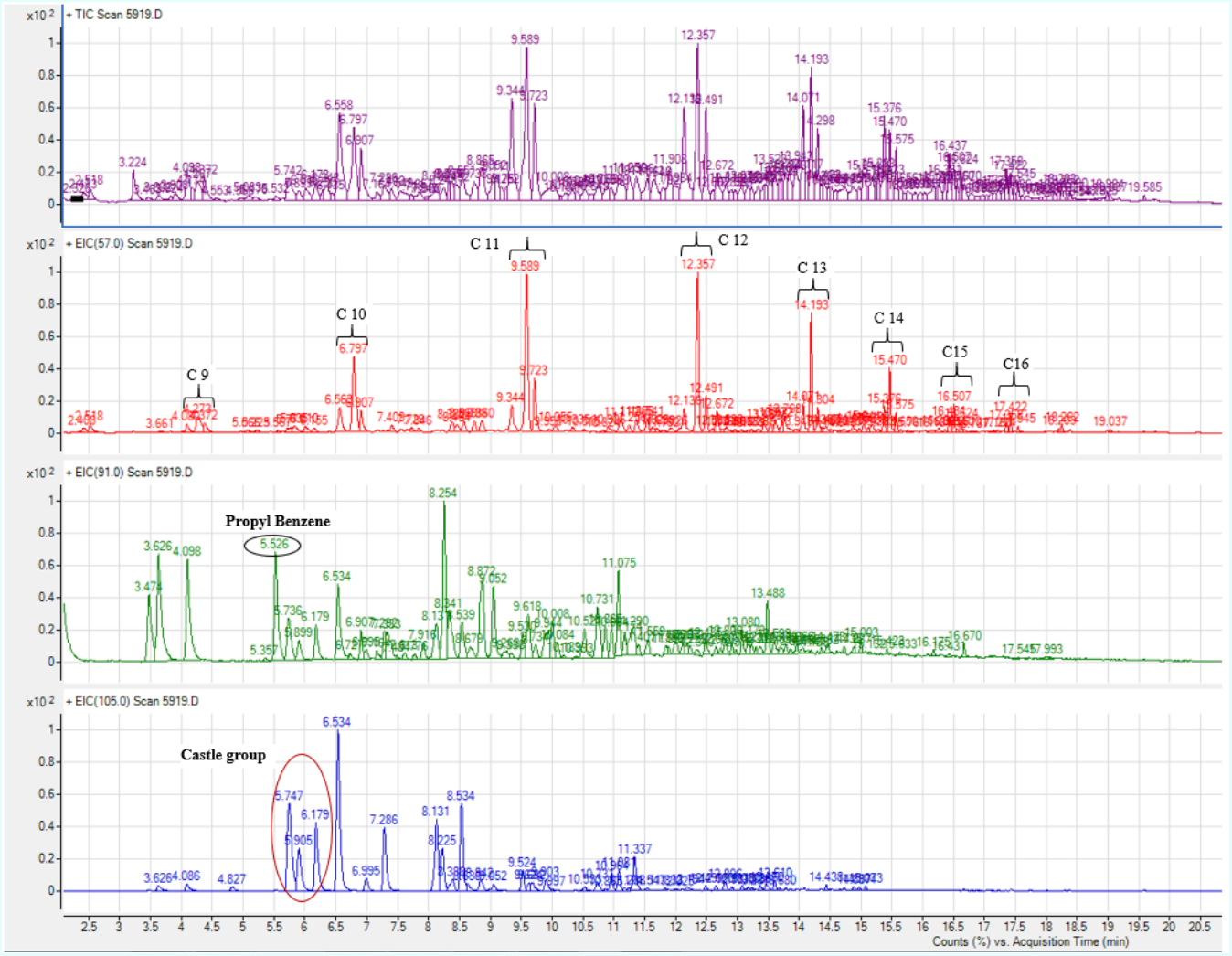


*Figure 4: EIP for sample spiked with 20 µl kerosene*

Table 2 further shows the comparison of the retention times of the target compounds for the 15 and 20 µl spiked samples with reference to the control chromatograms in Figure 1.

*Table 2: Comparison of the target compound retention times of 15 and 20 µl spike samples*

| Compound | Retention time/min | | | | | |
|---|---|---|---|---|---|---|
| | 0.1% Kerosene Reference | Neat Sample | Burnt without Kerosene | Pyrolysed Sample | Burnt with Kerosene | |
| | | | | | Spiked volume 15µl | Spiked volume 20µl |
| Nonane | 4.279 | - | 4.268 | 4.291 | 4.269 | 4.270 |
| Propyl cyclohexane | 4.876 | - | - | - | - | - |
| n-Propylbenzene | 5.530 | - | - | - | 5.537 | 5.526 |
| 1-ethyl-3-<br>1-ethyl-4- | 5.751 | - | 5.753 | - | 5.753 | 5.747 |
| 1,3,5-trimethylbenzene | 5.901 | - | - | - | 5.905 | 5.905 |
| 1-ethyl-2- | 6.183 | - | - | - | 6.179 | 6.179 |
| 1,2,4-trimethylbenzene | 6.537 | - | - | - | 6.534 | 6.534 |
| Decane | 6.797 | 6.803 | 6.791 | 6.790 | 6.791 | 6.797 |
| 1,2,3-trimethylbenzene | 7.286 | - | - | - | 7.286 | 7.286 |
| n-Butylcyclohexane | 7.550 | - | - | - | - | - |
| Trans-Decalin | - | - | - | - | - | - |
| Undecane | 9.589 | - | 9.583 | 9.581 | 9.583 | 9.589 |
| 1,2,3,5-tetramethylbenzene | 10.016 | - | - | - | 10.020 | - |
| n-Pentylcyclohexane | 10.409 | - | - | - | - | - |
| Dodecane | 12.357 | 12.362 | 12.351 | 12.346 | 12.351 | 12.357 |
| n-Hexylcyclohexane | - | - | - | - | - | - |
| Tridecane | 14.119 | - | 14.193 | 14.199 | 14.193 | 14.193 |
| n-Heptylcyclohexane | - | - | - | - | - | - |
| Tetradecane | 15.470 | 15.481 | 15.470 | 15.476 | 15.470 | 15.470 |
| n-Octylcyclohexane | - | - | - | - | - | - |
| Pentadecane | 16.510 | - | 16.513 | 16.513 | 16.513 | 16.507 |
| n-Nonylcyclohexane | - | - | - | - | - | - |
| Hexadecane | 17.431 | - | 17.422 | 17.422 | 17.422 | 17.422 |

## Conclusion

Since, EIP for 15 µl sample showed both castle group and relevant alkanes with Gaussian pattern, it could be interpreted as positive response.

Therefore, it is recommended that in order to identify trace levels of kerosene, significant consecutive C3 aromatics (castle group) must also be considered along with target compounds of kerosene to avoid misinterpretation of results.

## References


[1] *Stauffer E. Concept of pyrolysis for fire debris analysts. Science & Justice. 2003; 43(1):29-40.*

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[4] *ASTM International. ASTM E1618 - 14 Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry [Internet]. Astm.org. 2020 [cited 20 August 2020]. Available from: https://www.astm.org/DATABASE.CART/HISTORICAL/E1618-14.htm*

# Identification Of HMX by Direct Analysis in Real-Time Time-of-Flight Mass Spectrometry

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## Abstract

HMX (cyclotetramethylene tetranitramine) is a nitroamine high explosive with multiple nitro groups in the structure. This study aims to develop a convenient surface analytical method for the quick identification of this explosive by a direct analysis in real-time (DART) time-of-flight (TOF) mass spectrometer. The possible ionization mechanism and fragmentation pathway under DART was proposed for each molecular or fragment ions of HMX, based on the in-source collision induced dissociation (CID) spectra acquired by a DART AccuTOF MS. This HMX compound tends to release the reactive nitro group in a radical form, thus the loss-of-nitro-group fragments as well as other nitro group adducts could be characteristic ions for the identification of such explosives. This DART-TOF MS method shows great potential for the quick identification of explosives residues from different samples exposed to the blast scene and can be widely used for the criminal material evidence.

## Introduction

HMX has been widely used worldwide for both military assault and communal facilities reconstruction. It has multiple electrophilic nitro groups in the structure, which makes them very unstable at high temperature or encountering mechanical shock, and leads to tremendous destructive power. It is an urgent demand for the material evidence authentication to detect such highly unstable explosive residues in a public environment rapidly and accurately. However, such explosives-contaminated samples are hard to be prepared from different kinds of materials or dust at the blast scene, and cannot be ionized and identified easily by LC/MS system due to its thermal instability.

DART is a relatively new ionization technique that was first discussed by Cody et al. in 2005 [1], which takes advantage of the production of metastable gas atoms generated from a glow discharge plasma in a heated gas stream. The heated gas stream interacts with the sample and depending on operational conditions, a number of ionization pathways can occur [2]. This technique allows for sample introduction directly into the gas stream between the source and the mass spectrometer inlet, providing a way to analyze a sample under atmospheric conditions using high temperature and high voltage, without a high potential. It could be a softer ionization mode than electron ionization (EI), but can give better ionization efficiency for a couple of compounds that electrospray ionization/ atmospheric pressure chemical ionization/ atmospheric pressure photoionization (ESI/APCI/APPI) cannot ionize well [3,4].

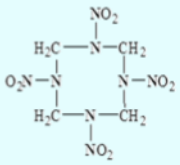
High-resolution TOF-MS systems are capable of measuring the accurate molecular weight, thus calculating the possible elemental composition. However, with better mass accuracy of below 1ppm, the number of possible formulae identified can still be more than one. In order to get a confirmable identification result and better insight into the ionization behavior of explosives in ambient circumstance, we try to elucidate the ionization mechanism of HMX under DART, and propose the potential in-source collision induced dissociation (CID) fragmentation pathway for each compound. By integrating mass accuracy and fragmentation information, high resolution analytical technique could help us to identify explosives rapidly and confidently. DART-TOF MS shows great potential for the quick identification of explosives residues from different sample matrices recovered from the blast scene.

## Experimental Methods

### *Chemical and Reagents*

HMX explosive standard was purchased from Xi'AN Modern Chemistry Research Institute and listed in Table 1. Acetone (Sinopharm Chemical Reagent Co., Ltd) was used for explosives' dilution and extraction from standards or complex sample matrix.

*Table 1. List of HMX used in this study as well as other physical and chemical properties*

| Abbreviation | IUPAC Name | Formula | Structure | Melting Point | Reactive Group |
|---|---|---|---|---|---|
| HMX | cyclotetramethylenete tranitramine | $C_4H_8N_8O_8$ |  | 278℃ | $-NO_2$ |

***Instrument and Parameters***

All data were acquired on an AccuTOF MS high resolution TOF MS (Electron JEOL Company of Japan, JMS - T100LP) with a DART Ion Source (U.S Ion Sense Company). See Figure 1 for the overview of instrument configuration. The DART parameters and control were operated using the DART SVP program.

*Figure 1. DART- AccuTOF mass system*

Several DART detection conditions were optimized to get the best ionization sensitivity. Helium gas was used as the ionizing gas with a flow rate of 2.5 L/min for the compound. The heater temperature is 200°C, while 3999V optimal for sample. The relatively higher grid voltage also benefits the production of characteristic fragments for further identification. The AccuTOF MS performed scan from m/z 50 to 500 Da in negative ion mode with resolving power greater than 6000. The distance between DART source outlet and MS orifice was 25 mm. Needle voltage was 4000 V, the outlet deflecting voltage was -100 V.

***Sample Preparation***

HMX was dissolved and diluted to 1 µg/mL by acetone individually. The explosives powders or explosives residues from contaminated sample was extracted with acetone and diluted to appropriate concentration before analysis. This technique allows for sample introduction directly into the gas stream between the source and the mass spectrometer inlet, providing a way of analyzing samples under atmospheric conditions using high temperature.

***Data Processing***

Mass peak shape calibration for DART-TOFMS spectra was performed via sCLIPS function in MassWorks (Cerno Bioscience, USA), then elemental composition was determined by the same software. Typically, 5 mD of mass tolerance, 15% isotope pattern intensity tolerance and possible elements C (0 ~ 10), H (0 ~ 20), O (0 ~ 20), and N (0 ~ 20) were used for calculation.

## Results & Discussion

***HMX Standard Ionization and In-Source CID Fragmentation by DART***

Three anion ions at nominal m/z 342 $[M+NO_2]^-$,358 $[M+NO_3]^-$ and 398 $[M+C_2H_4N_3O_2]^-$ can be observed simultaneously from negative TOF MS spectrum (Figure 2) with the standard. In negative mode, we can find multiple fragment ions as well as several significant adducts from the TOF MS scan. Based on the calculation of the elemental composition, we find the fragmentation behavior of HMX is similar to that reported by Sigmen, et al., as the thermal decomposition process previously [5]. There are several common fragments, like nominal m/z at 102 $[C_2H_4N_3O_2]^-$, can also be observed from GC/MS with negative chemical ionization (NCI), but no significant molecular ions or adducts are produced due to the high electron ionization voltage [6]. It's the first time that we get so many characteristic ions under DART negative mode [7], indicating that DART can ionize such nitramine explosives softer without dissociating the structure too much. In the case of HMX, we assume that radical $\bullet NO_2$ and $\bullet NO_3$ can be released from multiple tertiary amine site when thermal desorption, then ring cleavage and further

dealkylation from the tertiary amine group can generate the major fragments of m/z 102, corresponding to $[C_2H_4N_3O_2]^-$, as illustrated in Figure 3. The deprotonation of parent compound may subject to the same dealkylation, that's why we can also get the high abundance of $[M+NO_2]^-$, $[M+NO_3]^-$ and $[M+C_2H_4N_3O_2]^-$ adducts at the same time. The radical $\bullet NO_2$ is very active and can easily react with $O_2$ and $H_2O$ in the air during the transient ionization moment, thus forming other fragment or adduct.

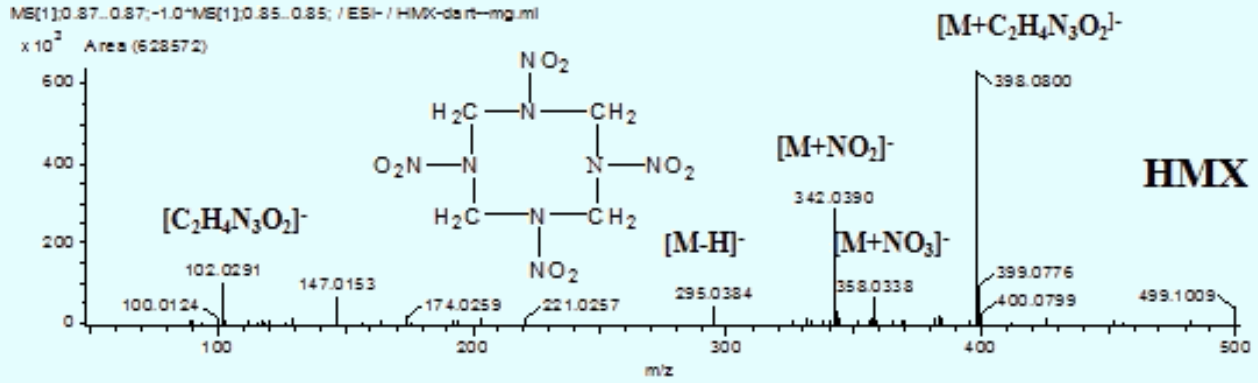



*Figure 2. In-source CID TOF MS spectrum of HMX under DART negative mode by AccuTOF*

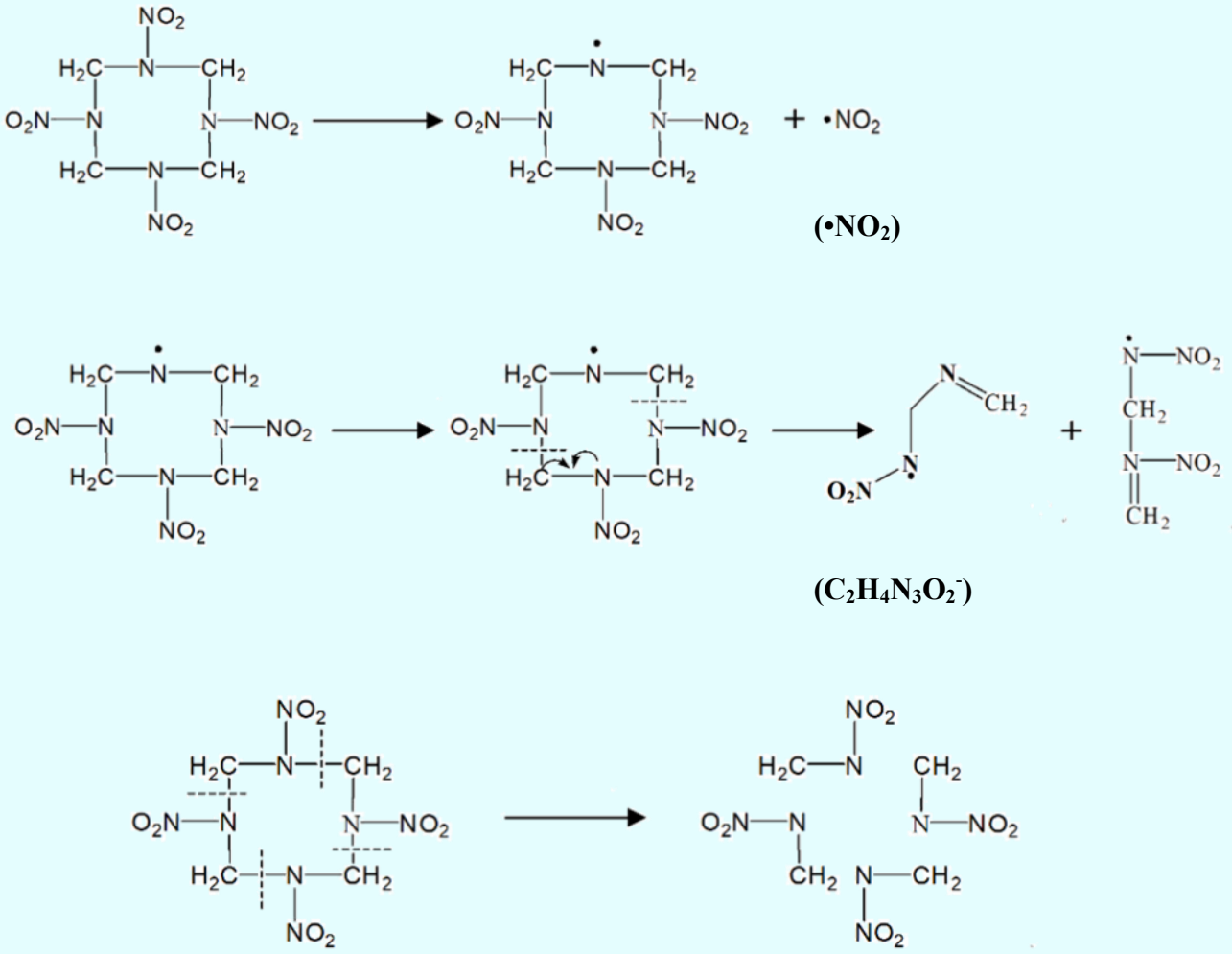



*Figure 3. Proposed fragmentation pathway for the radical anions [M+ NO2]•- (m/z 342) and [M+ C2H4N3O2]•- (m/z 398) of HMX.*

## Conclusions

To investigate the ionization mechanism of HMX using DART, three significant adduct ions namely m/z342 $[M+NO_2]^-$, 358$[M+NO_3]^-$ and 398$[M+C_2H_4N_3O_2]^-$, are found to be formed from radical ions generated from the fragmentations of HMX. Their potential in-source CID fragmentation pathways are proposed in this paper, and the detection limit for such ions is found to be 0.05ug/ml. Nitro group shows great reactivity when undergoing thermal decomposition and tends to be released in a radical form. These characteristic ions, including loss of nitro group fragments, as well as other nitro group adducts, could benefit the identification of such explosives.

DART-TOF MS is a convenient surface analysis technique for the quick identification of HMX from different kinds of sample matrix with very simple sample treatment, which may be hard to be analyzed by using traditional LC/MS sample preparation workflow. It was found that acetone can enhance the ionization sensitivity of these compounds under DART, and the spectra acquired are much more useful and simpler than EI or other ionization mode for elucidation. This paper illustrated that the DART-TOF MS is an effective technique for the detection of trace explosives, such as HMX, at the explosion scene.

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# Cannabis Use? Or Something Legitimate?

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In Singapore, consumption of controlled drugs is an offence. Urine samples procured by the law enforcement agencies are typically screened using an immunoassay technique as a preliminary test prior to sending to our Laboratory for further testing. Immunoassay techniques are known to be fast and efficient in screening for classes of drugs. However, if a compound other than the controlled drug present in the urine cross-reacts with the antibody, a false positive result will be obtained. In the Laboratory, urine with a positive immunoassay result must be further tested by a second technique, such as, gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS), to confirm if a controlled drug is present.

Three urine samples from a suspected cannabis abuser (the subject) procured on different days were submitted to the Laboratory for further analysis after preliminary screening showed positive for cannabinoids using CEDIA[1] immunoassay in Beckman Coulter AU 480/680 at the law enforcement agency site. However, the urine samples were screened negative for cannabinoids in the Laboratory using KIMS[2] immunoassay in Cobas c501. Further confirmation using GC-MS in Selected Ion Monitoring (SIM) mode showed negative for 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in the all three urine samples, and hence, the positive screening by CEDIA was not due the presence of THC-COOH in urine.

Further investigation on the case revealed that the subject was on the medication – efavirenz. Efavirenz is an antiretroviral medication used to treat Human Immunodeficiency Virus infection (HIV). A dose of 600 mg once daily is recommended for adult [1]. Efavirenz produces several metabolites in the human body, with 8-hydroxy efavirenz and 8-hydroxy efavirenz glucuronide being the major metabolites excreted in urine [2-5]. 8-Hydroxy efavirenz is not known to cross-react with cannabinoids immunoassays in urine. However, the 8-hydroxy efavirenz glucuronide is known to cross-react with some cannabinoids immunoassays in urine [3-5].

In this study, aliquots of the three urine samples were hydrolysed by β-glucuronidase from abalone *(Haliotis Rufescens)*. Screening tests were performed on both untreated and enzyme treated urine samples using Cobas c501 and Beckman Coulter AU 480/680 for comparison. The enzyme treated urine samples were also analysed using liquid chromatography coupled with a Thermo Scientific Q-Exactive Hybrid Quadrupole-Orbitrap mass spectrometer (LC-Obitrap MS) operating in Full MS and data-dependent $MS^2$ ($FS\text{-}ddMS^2$) negative scanning mode for the presence of 8-hydroxy efavirenz using the reference material, rac-8-hydroxy-efavirenz acquired from Clearsynth®.

| Instrument | Cobas c501 | | Beckman Coulter AU 480/680 | |
|---|---|---|---|---|
| Sample number | Before enzyme treatment (ng/ml) | After enzyme treatment (ng/ml) | Before enzyme treatment (ng/ml) | After enzyme treatment (ng/ml) |
| Sample 1 | 4 | 0 | 70.00 | 2.51 |
| | 0 | 0 | 73.99 | 3.02 |
| Sample 2 | 2 | 0 | 84.51 | 4.27 |
| | 2 | 0 | 76.55 | 3.76 |
| Sample 3 | 5 | 0 | 92.39 | 4.25 |
| | 7 | 0 | 95.90 | 6.20 |

*Table 1. Screening results of the urine samples in duplicate by Cobas c501 and Beckman Coulter Au480/680*

[1] Cloned Enzyme Donor ImmunoAssay
[2] Kinetic Interaction of Microparticles in Solution

As shown in Table 1, no presumptive presence of cannabinoids in urine was found before or after enzyme treatment using KIMS in the Cobas c501. While for screening using CEDIA in the Beckman Coulter AU 480/680, the results were positive (cut-off at 50 ng/ml) before the enzyme treatment and became negative after the urine samples were treated with the enzyme.

KIMS is not known to have cross-reactivity with the 8-hydroxy glucuronide conjugate of efavirenz. However, CEDIA reagents are known to cause false positive cannabinoids screening in patients using efavirenz, possibly due to the presence of 8-hydroxy efavirenz glucuronide [3-4].

The result of analysis of the enzyme treated urine samples using the LC-Orbitrap-MS (Figure 1) indicates the presence of 8-hydroxy efavirenz in the urine samples by comparing the retention time and ddMS$^2$ spectrum of the 8-hydroxy-efavirenz reference material (Figure 2).

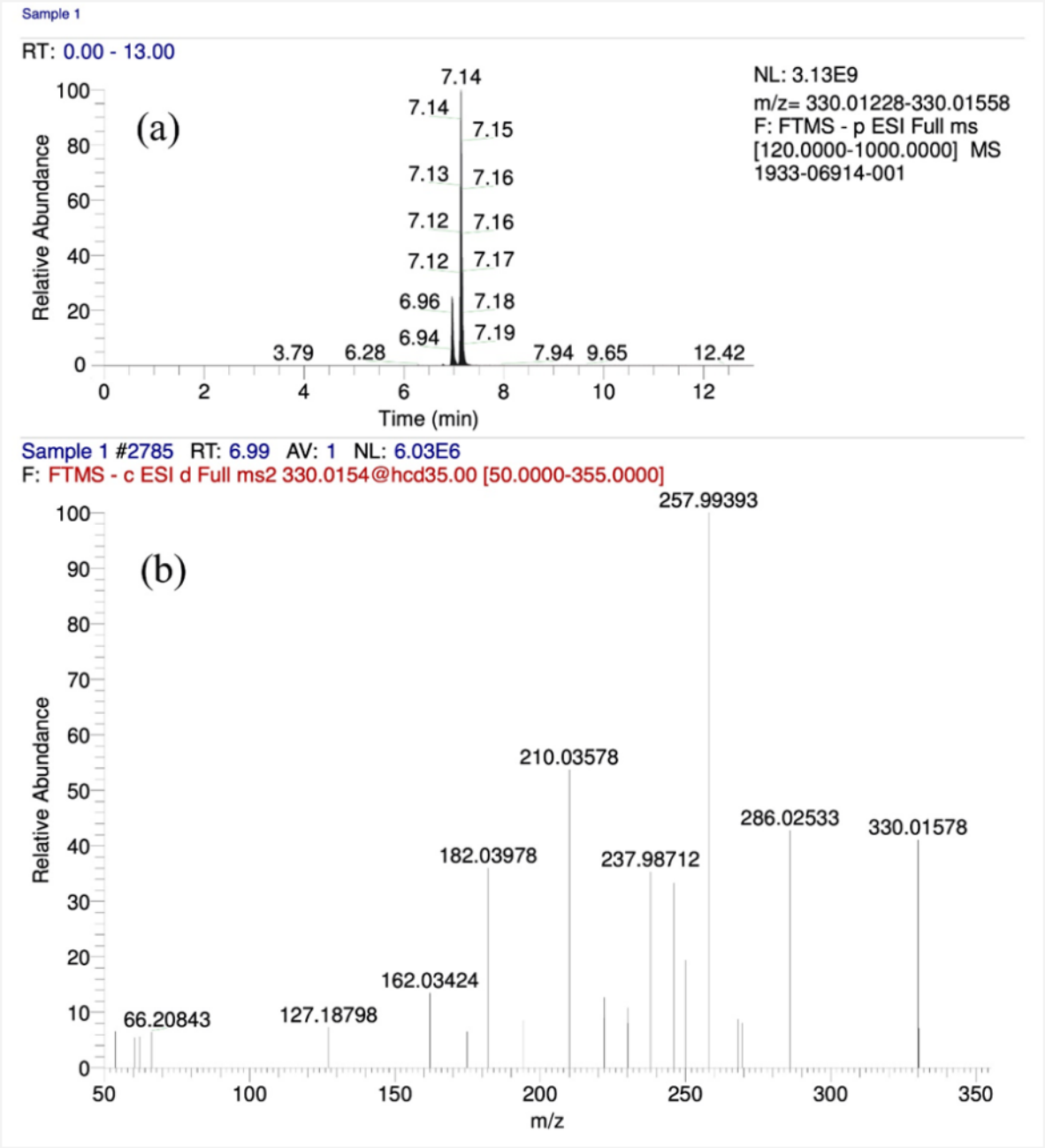


*Figure 1. (a) Chromatogram and (b) ddMS$^2$ spectrum of a urine sample submitted for cannabinoids analysis.*

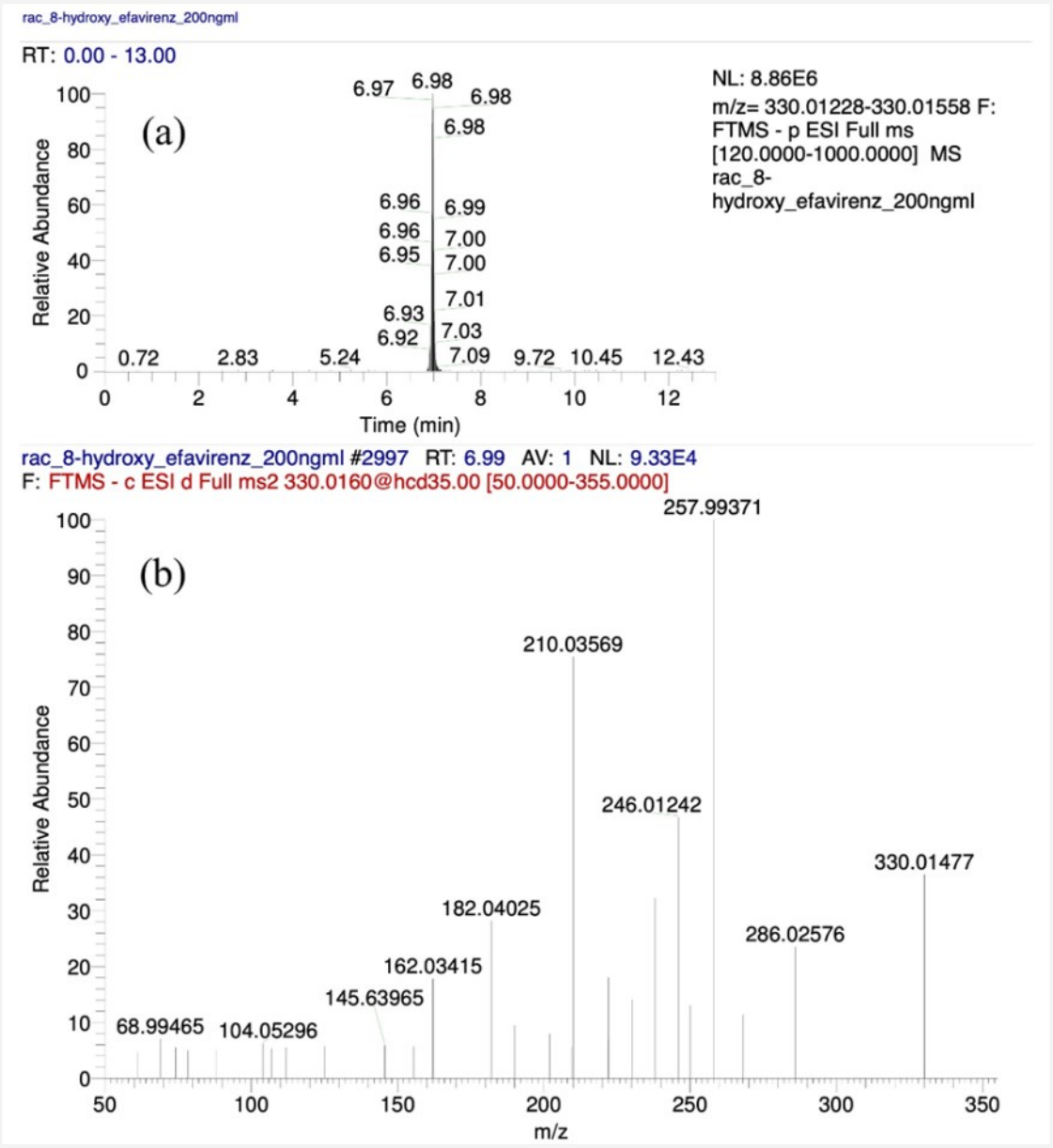


*Figure 2. (a) Chromatogram and (b) ddMS2 spectrum of rac-8-bydroxy efavirenz at 200 ng/ml in LC-orbitrap-MS negative mode*

In summary, the study shows that consumption of efavirenz can result in false positive cannabinoids screening in the CEDIA but not KIMS immunoassays. Therefore, chromatographic/mass spectrometric confirmatory test, such as GC-MS, must be used to confirm the presence/absence of the cannabis metabolite, THC-COOH, in urine samples after they are screened positive for cannabinoids by immunoassays.

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# A Case Of Video Content Analysis in Forensic Science

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## Introduction

Video content analysis is based on the understanding and judgment of video content analysis technology. Due to the video image differ in thousands ways, identification requirements are complicated, which requires us video examiners, from the perspective of the video image itself, the research on the rules of its inherent essence, depth excavation the message coming out of the video image is our job.

Video data contains rich semantics such as characters, scenes, objects, behaviors and stories, which can realize the content analysis of video data, which has profound theoretical research significance and strong practical application value [1]. Video content analysis mainly analyzes video internal resources and video external resources [2].

We use an industry standard named Technical specification for event sequence inspection in video (GA/T 1020-2013).

## Background Information

In a case which happened in Gansu province in 2016, an old woman fell (one foot on the bus, another foot on the ground) while she was onto the bus, and died after being rushed to hospital. The identification requirement is whether the bus move or not when the old woman onto the bus.

## Materials and Methods

*Instrumentation*

The entrusted unit sent 4 videos of the bus monitor, and we selected the most valuable paragraph for analysis. See Table 1 for the attributes of the video. We use a sharp knife cut function depending on the OVIT player to intercept picture shows time at 10:45:15-10:45:35 of video, a total of 482 frames. As shown in Figure 1 and Figure 2. The old woman get on the bus at the time 20 seconds, as shown in Figure 3.Video picture shows the bus front door view, which shows the front door of the bus, the handle of the door, the coin box, and the outside trees and pavement of the bus.

| No. | Video Attribute | Value |
|---|---|---|
| 1 | Filename | 甘B0701300000000-160131-103936-105436-00n402000000.264 |
| 2 | MD5 Value | 70545851AB5858CB45EC6DDA1631E608 |
| 3 | Video Size | 140,608KB |
| 4 | Duration | 13'29" |
| 5 | Frame Size | 704×576 |
| 6 | Frame Rate | 20fps |

*Table1 Inspection of material properties*


*Figure 1: Start Frame*


*Figure 2: End Frame*


*Figure 3: The Frame of old women get on the bus*

*Analysis*
*(a) Select test point*

Select the top of the screen and the right junction of the tree to test point 1, the inside of the bus is located near the top corner of the driver to test point 2, as shown in Figure 4.

*(b) Measurement*

VISystem was used to measure the X axis value of test point 1 and test point 2 from start frame to end frame, Figure 5 showed how pixels were measured using Photoshop. The image top left corner is the origin, made a curve as follow, see Figure 6 (The x-coordinate is the frame number, and the test point coordinate value of the X-axis is the y-coordinate.)

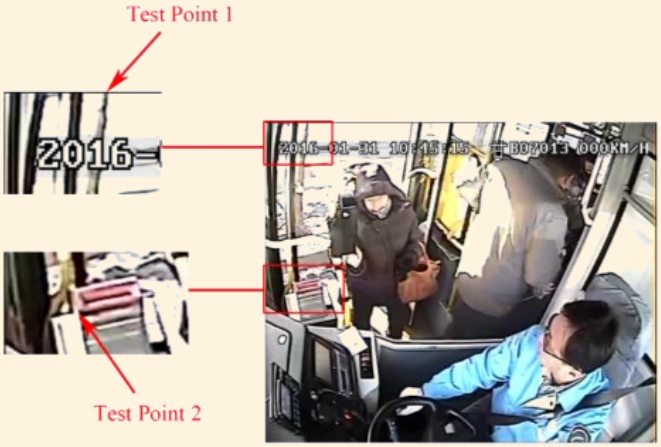


*Figure 4: Position of the test point*

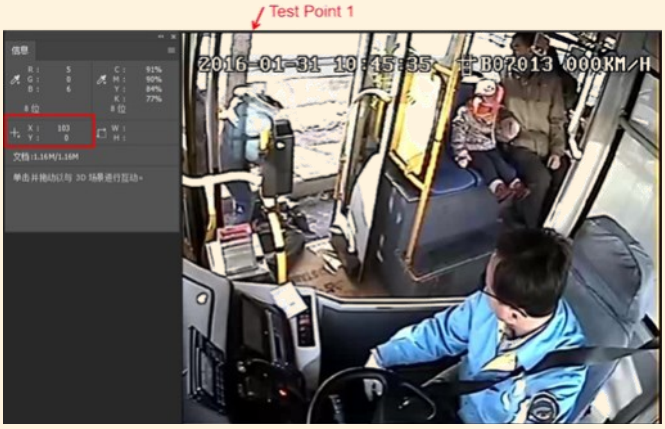


*Figure 5: How pixels were measured using Photoshop*

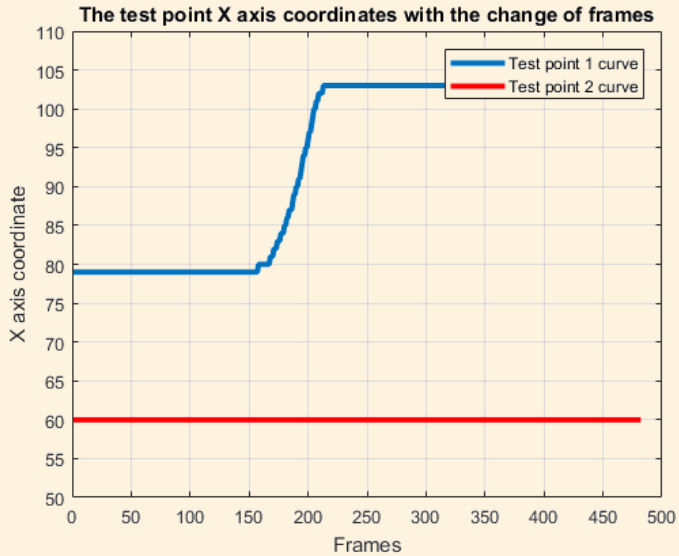


*Figure 6: The test point X axis coordinates with the change of frames*

*(c) Analysis of the curve*

Because the bus inside the test point relative to the buses are fixed point 2, test point 1 is outside of the trees, if there is a moving bus, then the distance of the test points 1 and 2 will have change, if there is no movement, the distance of the test points 1 and 2 should remain the same.

According to the measurement results, from frame 1 to 157, the test point 1 is unchanged. From frame 158 to 212, the test point 1 was significantly changed, and 24 pixels were moved, and the approximate distance of moving on the screen was the diameter of the trees. Frame 213 to 482, the test point 1 is unchanged. According to the formula v=S/t, it is assumed that the diameter of the trees is 24 centimeters, 158 frames to 212 frames are 54 frames (about 2.7 seconds), and the time of the bus is estimated to be 0.089 m/s.

*Workflow*

The workflow in this case of video content analysis as follow Figure 7.

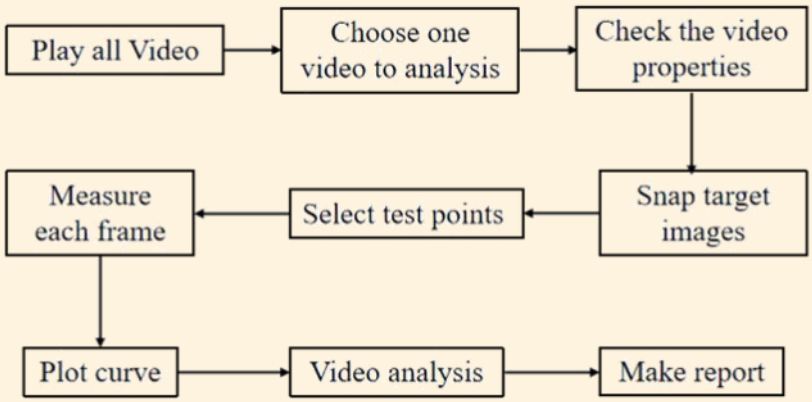


*Figure 7: The workflow of this case*

## Results and Discussion

According to the above analysis, video footage showed time at 45 minutes and 15 seconds to 10 at 45 minutes and 35 seconds, within the scope of the 158th frame to 212th frame period (about 2 seconds) relative movement between the tree and bus.

## Conclusion

Qualitative analysis, video content analysis are generally stronger subjectivity, descriptive language commonly used. In this paper, we used the method of video measurement, plotted a curve of the test point, the quantitative description the movement process of vehicle, more intuitive and the objective, provides the case broke strong evidence, is worthy of reference for colleagues.

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Case Study

# DNA Forensics In Insurance Fraud: A Case Study

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## Abstract

A person with an intention to defraud insurance companies created a crime scene by strangulation of his associate; put his body in a car which was rolled down the hill and made splash patterns of brownish blood on the road to simulate conditions of scuffle and robbery followed by death in a road accident. The forensic examination of the scene of crime and laboratory analysis of physical clues through DNA profiling helped to solve the mystery of crime and spoiled game plan of the person to defraud insurance companies and was put behind the bars for the homicide of his associate.

## Introduction

Ever since, the DNA technology has emerged gold standard in forensics [1], it helped in solving mysteries of crimes, role in ascertaining guilt or innocence of the accused, immigration problems [2,3], identification of unidentified bodies [4,5] and victims of disasters [6,7,8], wildlife crime [9,10] and so on. The presence of large blood splash pattern either at indoor crime scene or outdoor crime scene, more often than not, is construed as a case of offence against body viz. homicide [11], assault [12], or also due to injuries suffered in road accident cases.

## Case Report

A person who wanted to make quick buck planned strategy to defraud insurance companies and bought different life insurance policies worth millions of US Dollars (INR 3 crore) over a **s**pan of time. Accordingly, he created an outdoor crime scene with his own blood after travelling in his car about 350 km away from his house and entered the territory of another state in forest area. He had lured a known acquaintance and consumed liquor together. When the associate was completely drunk, he strangulated the acquaintance, poured acid over the face of deceased (Figure-1) to conceal identity and then put his own unique identification card (Aadhar card issued by the government authorities to Indian nationals) and bank documents, bank passbook into pocket of the deceased, placed the body of the deceased in the driver's seat and then, the vehicle was thrown about 40-50 meters down the hill from the road to make a case of road accident to mislead the investigation (Figure-2). Also, he poured blood on the puckka road and created blood splash patterns to look like a case of scuffle and robbery on a road passing through forest area away from the main habitation (Figure-3).

After observing the blood splash patterns on the road and the mangled car in the forest area, the road user informed the police about the road accident. The police could not identify the deceased as the face was found partially charred. The police initially registered a case of road accident, believing that the driver was not accustomed to hill driving as the number plate of car indicated origin from bordering plain terrain.

Later on, forensic expert's visit to the crime scene led to recovery of abandoned plastic vial in the bushes about 30 feet away from the spot on the roadside and reconstruction of the scene of crime revealed that clues present on the deceased's body, on the mangled car and on the road were not consistent with a case of road accident. Further, investigations on the basis of bank passbook and Aadhar card helped the police to reach the owner of the car who was absconding from his house, but was finally arrested. The police collected the physical evidences from scene of crime like blood stains, plastic vial containing brownish liquid at 30 feet from the spot, blood stains from the mangled car, post-mortem blood from the deceased's body and blood sample of suspect on FTA card and all were sent for analysis to the forensic laboratory.

DNA isolation from blood of deceased and blood stain lifted at 30 feet from the spot was done with magnetic bead based method Qiagen EZ1 Advanced XL BioRobot [13] while DNA purification from the FTA card was done as per method given by Sahajpal et al.[14]. The amplification of isolated DNA was done with PowerPlex®21 System (Promega Corporation, U.S.A.) as per protocol mentioned in the kit [15].

*Figure-1: A front view of deceased's face*

*Figure-2: A view of mangled car in the forest area*

*Figure-3: Splash patterns on the road*

Capillary electrophoresis of DNA was done with ABI 3130 Genetic Analyzer (Applied Biosystems, U.S.A.) and genotyping was carried out using GeneMapper® ID Software Version 3.2.

The DNA analysis revealed that the profile obtained from blood stain lifted at 30 feet from the spot matched with the profile obtained from blood sample of suspect on FTA card, but was different from the DNA profile of the deceased found in the driver's seat. The genotypes of DNA obtained from blood sample of the deceased, blood sample of the suspect on FTA card and blood sample in a plastic vial lifted at 30 feet from the spot is given in table 1 and their electropherograms are shown in figure-4, figure-5 and figure-6 respectively. On the basis of the analysis of forensic evidences based on DNA profiles, the presence of the suspect on the spot was proved. Ultimately, the suspect was arrested for the murder and could not defraud the insurance companies.

*Table-1*
*Genotypes of blood samples (Deceased, suspect and blood stain from the spot)*

| Genetic markers | Blood sample of deceased | Blood sample of suspect on FTA card | Blood stain lifted at 30 feet from the spot |
|---|---|---|---|
| | Alleles | Alleles | Alleles |
| Amelogenin | X,Y | X,Y | X,Y |
| D3S1358 | 15,15 | 16,17 | 16,17 |
| D1S1656 | 16,16 | 11,17.3 | 11,17.3 |
| D6S1043 | 12,17 | 13,19 | 13,19 |
| D13S317 | 11,13 | 11,12 | 11,12 |
| Penta E | 10,13 | 10,18 | 10,18 |
| D16S539 | 8,11 | 9,10 | 9,10 |
| D18S51 | 14,14 | 13,14 | 13,14 |
| D2S1338 | 17,23 | 18,20 | 18,20 |
| CSF1PO | 12,12 | 11,12 | 11,12 |
| Penta D | 9,11 | 10,13 | 10,13 |
| TH01 | 6,9.3 | 6,7 | 6,7 |
| vWA | 17,18 | 16,18 | 16,18 |
| D21S11 | 29,32.2 | 28,29 | 28,29 |
| D7S820 | 11,11 | 8,9 | 8,9 |
| D5S818 | 10,11 | 11,11 | 11,11 |
| TPOX | 8,11 | 8,10 | 8,10 |
| D8S1179 | 8,13 | 11,14 | 11,14 |
| D12S391 | 19,22 | 19,21 | 19,21 |
| D19S433 | 13,13 | 13,16 | 13,16 |
| FGA | 23,25 | 22,23 | 22,23 |

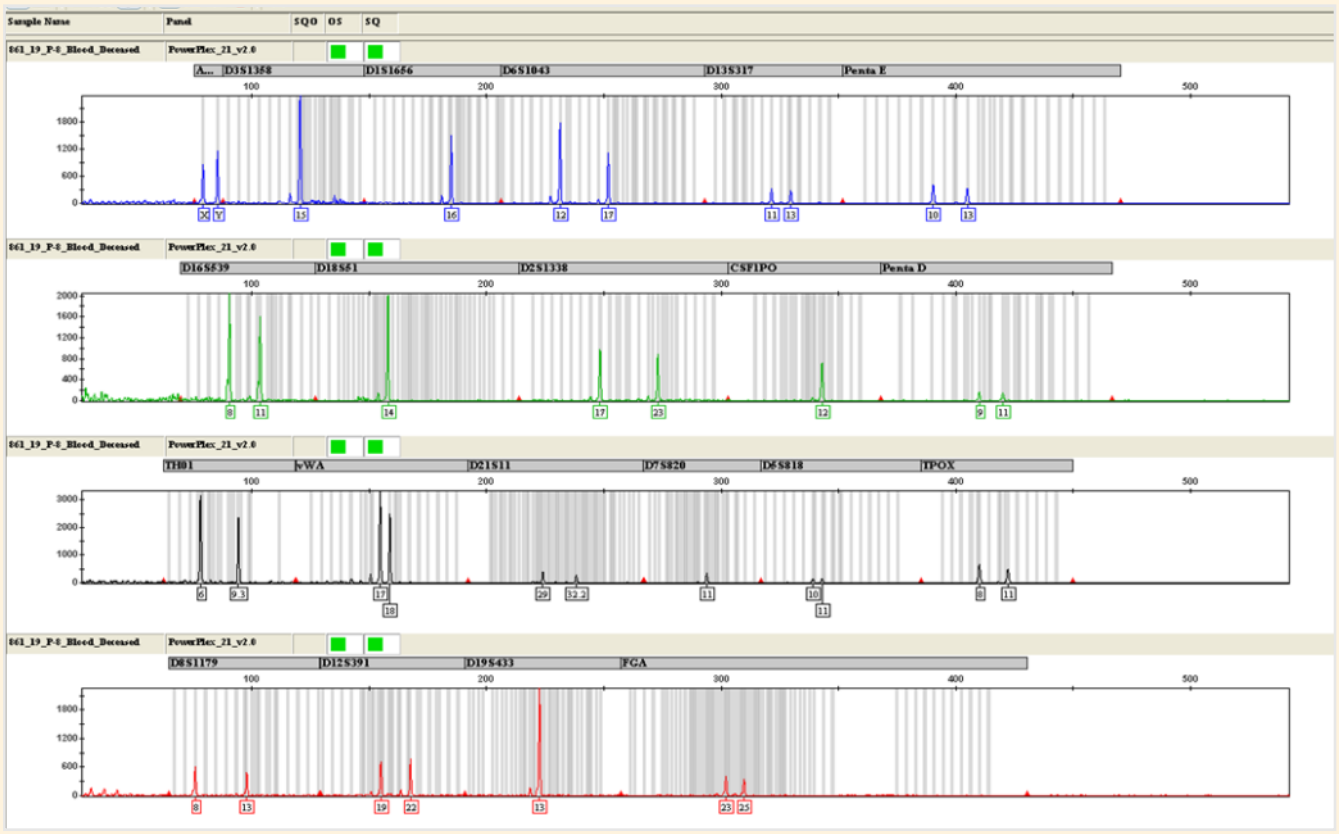

*Figure-4: Electropherogram of DNA isolated from blood sample of deceased person*

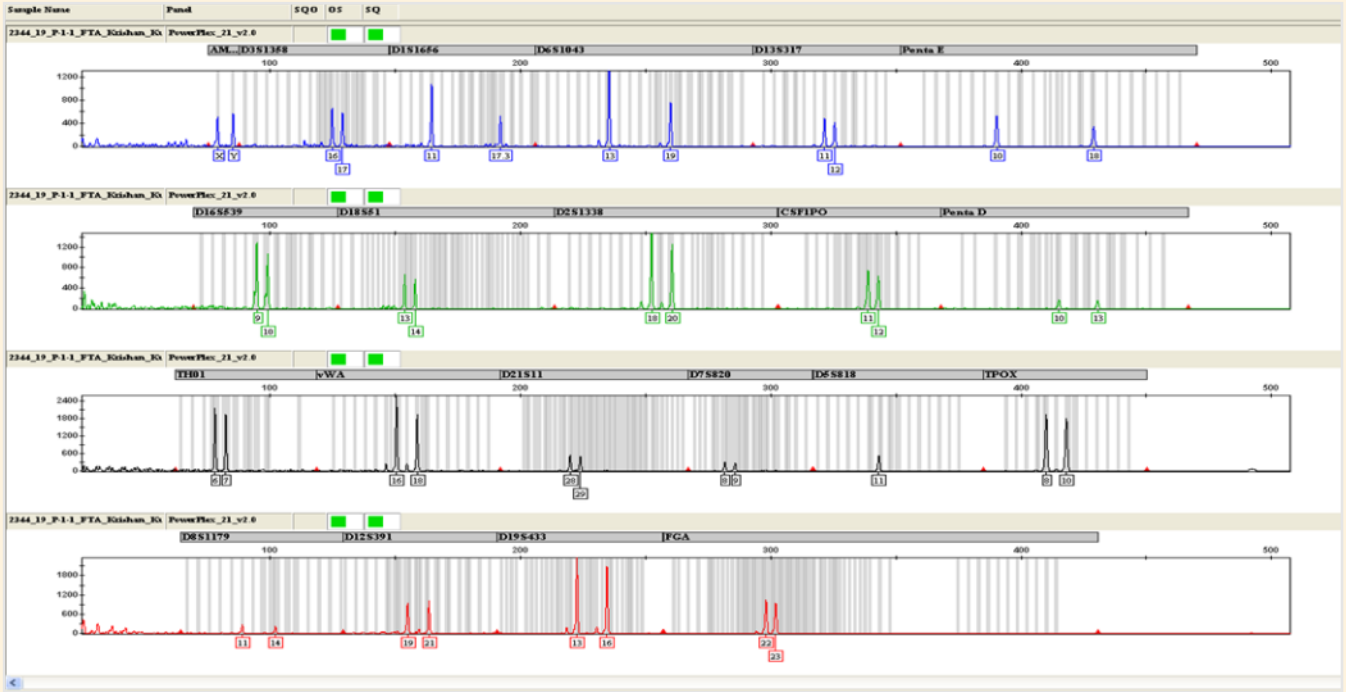

*Figure-5: Electropherogram of DNA isolated from blood sample of suspect on FTA card*

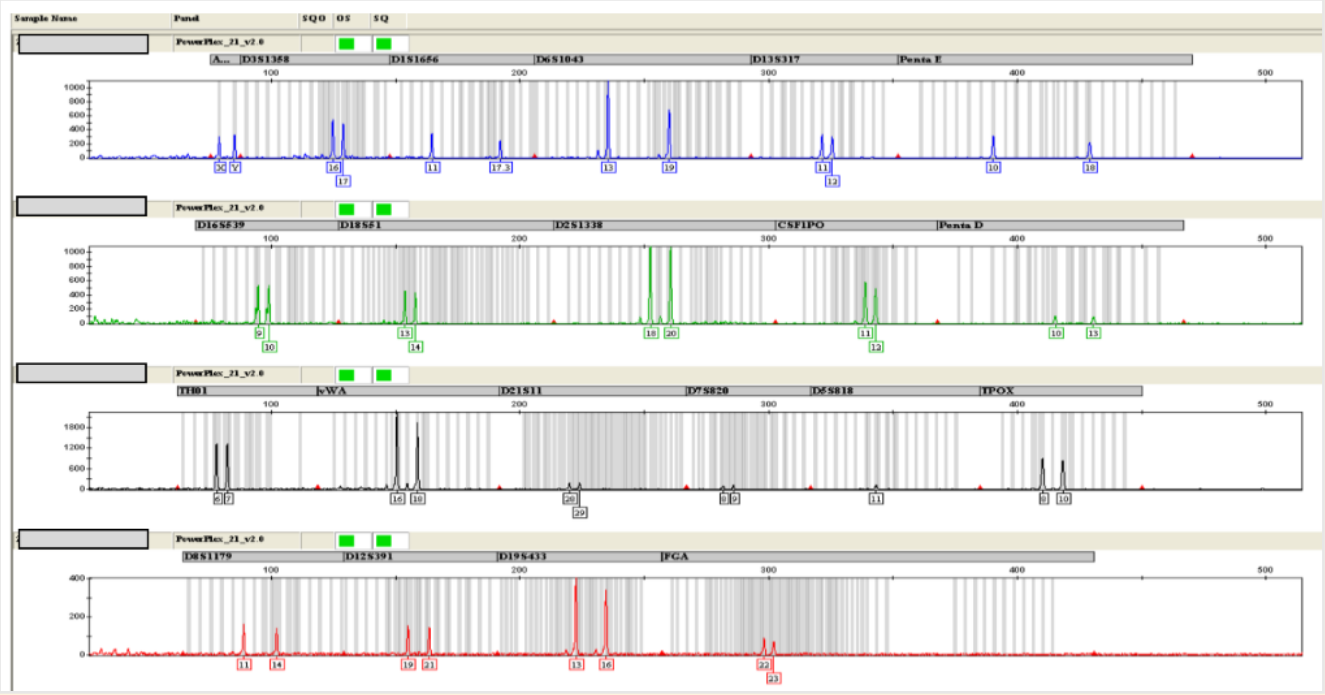

*Figure-6: Electropherogram of DNA isolated from blood stain lifted at 30 feet from the spot*

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# Case Study : Profiling of Seized Methamphetamine Synthesized by Reductive Amination Route

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## Introduction

Methamphetamine (MA) is the most abused drugs in Indonesia. Total MA seizure was about 17.9 tons in 2019 [1]. Based on our profiling study, the synthesis route of MA in Indonesia was dominated by Emde methods which used ephedrine/pseudoephedrine as precursor. For a long period, reductive amination route was not popular in Indonesia [2]. The last report was in 2010 where a clandestine laboratory of MA has been dismantled by NNB's investigators in Medan, Sumatera Utara. They synthesized MA from P2P via reductive amination route. Other chemicals found were methylamine, methanol and sodium borohydride. In this article, we report a new case of seized MA with unusual drug profile, *levo*-MA, which presumed to be synthesized by reductive amination of P2P.

## Case

Five kilograms of MA in the "QING SHAN" package were seized by Directorate of Interdiction of National Narcotics Board in Aceh Timur, February 2020 (Fig.1). One gram of sample was submitted to our laboratory for drug profiling purpose.

(a) 


(b) 

*Fig. 1. (a) Seized MA packaging; (b) MA sample*

## Materials and methods

- Reference standards of *dextro*-MA, *levo*-MA, *levo*-ephedrine and *dextro*-pseudoephedrine were obtained from Lipomed.
- Melting point was determined using Stuart® SMP10 Melting Point Apparatus.
- Microscopic analysis was observed under Meiji Techno Microscope HD1500M.
- Chiral analysis was performed by UPLC-PDA. 100 mg sample was recrystallized with chloroform and diethylether prior to the analysis.
  *Instrumentation*: The column was OSAKA SODA Column Chiral (4.6mm x 150mm, 5µm), temperature was set at 40°C. Buffer solution KH2PO4 20 mM/Acetonitrile (65/35) was used as mobile phase. Flowrate was 0.7 ml/min.
- Trace ephedrine analysis was performed by UPLC-PDA. *l*-ephedrine and *d*-pseudoephedrine standard solution were prepared at a final concentration 100µg/ml in water. 100 mg sample was dissolved in 2.5 ml water and 2 ml of solution was divided equally into two vials. One vial as a sample and another vial as sample mixed with standards by adding 50 µl of each standard solution.
  *Instrumentation*: The column was Shim-pack XR-ODS III C-18 (4.6mm x 150mm, 5µm), temperature was set at 40°C. Buffer solution KH2PO450 mM/Acetonitrile (98/2) was used as mobile phase. Flow rate was 0.3 ml/min.
- Impurity profiling was carried out on GC/MS using HP-5MS Column. Sample preparation and GC/MS condition were adopted from [3].

## Results and Discussion

Seized MA showed long orange needles under the microscope (Fig.2) with the value of melting point was 176–179ºC. Sample was composed of *levo*-MA as shown in Fig.3. Trace ephedrine analysis did not find *l*-ephedrine or *d*-pseudoephedrine in seized MA sample. It was presumed that manufacturer did not use ephedrine/ pseudoephedrine as precursor.

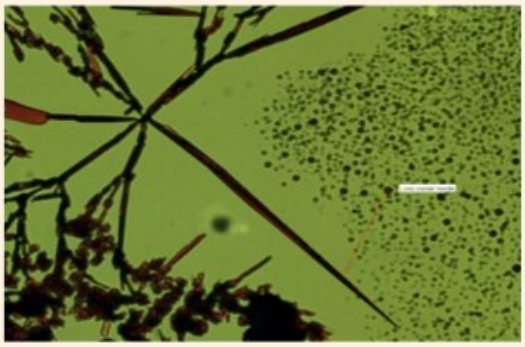

*Fig. 2. Characteristic of levo-MA in micro-scopic analysis (100x magnification)*

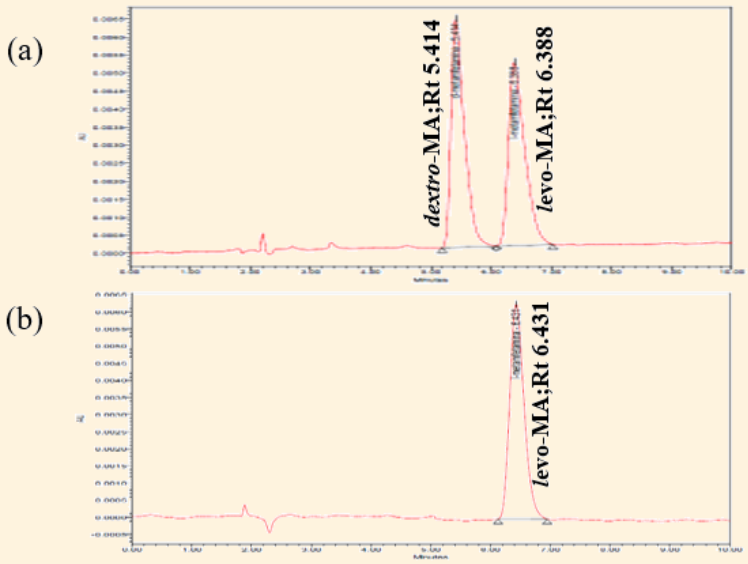


*Fig. 3. Chirality analysis of (a) mixed of MA standards; (b) MA sample*

A typical GC chromatogram of seized MA through acidic dan basic extractions are shown in Fig. 4 and 5, respectively. Diphenethylamine,alpha-methyl was found as impurity in both extractions. It was reported as impurity in P2P synthesize method [4]. In this case, P2P and P2P-ol were identified in acidic extraction only. P2P-ol was identified as route specific impurity for reductive amination route with P2P as precursor and this was reported by [3]. Other impurities attached in chromatogram previously presented by [3] are marked in bold. Impurity marked in Italic is mentioned in Ref [4].

This case was related to the trend of increased use of P2P as precursor of MA in East and Southeast Asia. China also reported an increasing of MA synthesized by reductive amination route from 2% in 2017 to 25% in 2018 [5]. The racemic mixture of MA as yield of P2P precursor, in this case, was presumed to be optically resolved to the *levo*-MA isomer with optical resolving reagent, such as tartaric acid [6].

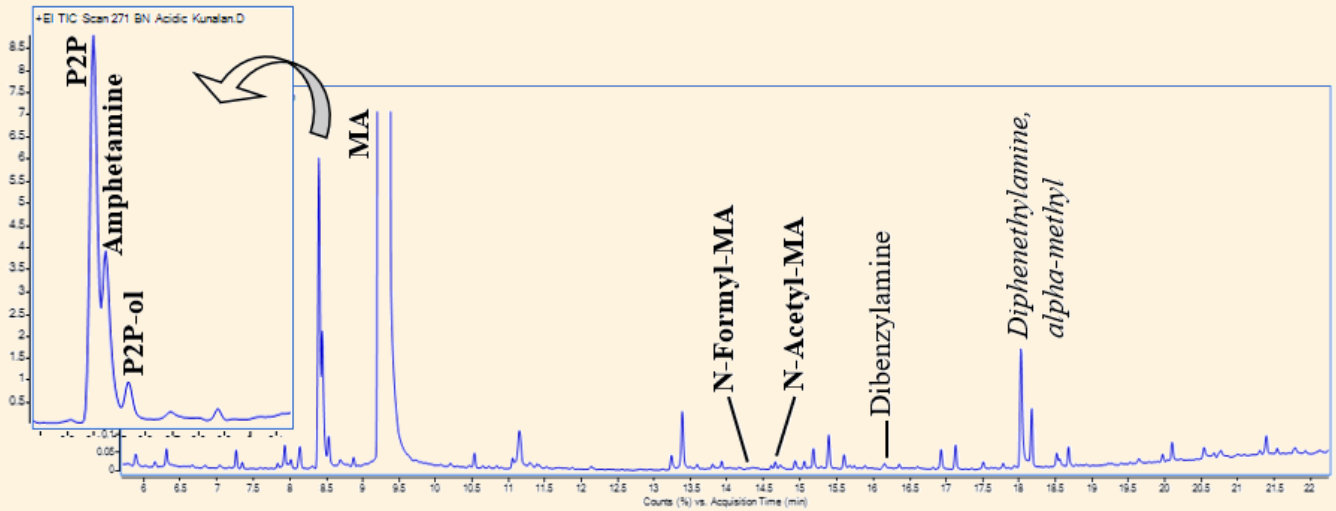


*Fig. 4. Typical TIC of seized MA under acidic extraction*

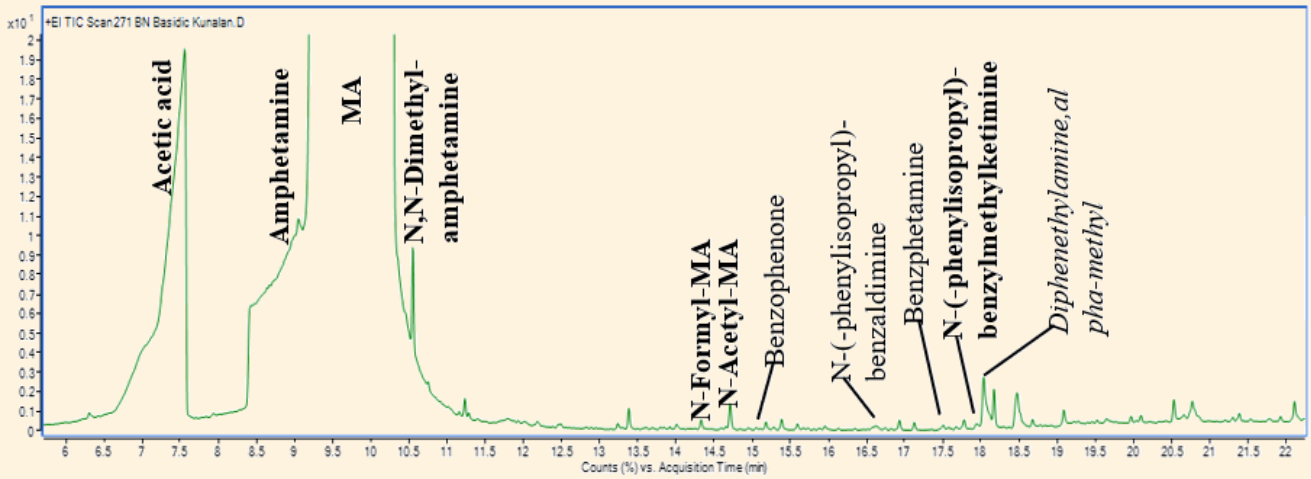


*Fig. 5. Typical TIC of seized MA under basic extraction*

## Conclusion

The authors concluded that from the profiling study, the seized MA was synthesized from P2P by reductive amination route. P2P-ol was detected and confirmed as route specific impurity for the reductive amination route with P2P as precursor.

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