INVESTIGATION OF CHLOROXYLENOL IN WATER ENVIRONMENTS OF HONG KONG AND THE USE OF FIRE EXTINGUISHER DRY POWDER TO DEVELOP LATENT FINGERMARKS BY MASS SPECTROMETRY

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Investigation of Chloroxylenol in Water Environments of Hong Kong and the Use of Fire Extinguisher Dry Powder to Develop Latent Fingermarks by Mass Spectrometry

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CERTIFICATE OF ORIGINALITY

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Deejay Mak Suen-yui

Abstract

Mass spectrometry (MS), a powerful analytical tool, was used to investigate issues in environmental science and forensic science, i.e., investigation of chloroxylenol in water environments of Hong Kong and investigation of the use of fire extinguisher dry powder to develop latent fingermarks. We aimed to understand and address the environmental challenges to protect the natural world, as well as to create new and novel protocols for the collection and analysis of latent fingermarks at crime scenes with the needs of criminal investigations.

Chloroxylenol, a halogenated phenolic compound, is an antimicrobial ingredient commonly used in antibacterial hand sanitizers and household disinfectants. Since the Coronavirus Disease-2019 (COVID-19), our habits and lifestyle behaviours have changed, causing a surge in worldwide demand for popular brands (e.g., Dettol, Walch and Ariel) of personal care products (PCPs) in which the main antimicrobial ingredient is chloroxylenol. As a result, enormous amount of chloroxylenol could be released into the aquatic ecosystem through drainage, posing toxicological threat to humans and aquatic animals, such as altered gene expression and histological lesions in freshwater fish. To investigate the extent of chloroxylenol contamination in Hong Kong, water samples were collected from two rivers (Tuen Mun River and Yuen Long Creek) and two sewage treatment plants (Stonecutters Island Sewage Treatment Works and Sha Tin Sewage Treatment

Works). Using solid phase extraction (SPE) for sample preparation and ultra-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC-ESI/MS/MS) for identification and quantification, concentrations of chloroxylenol in water samples were determined. Diurnal studies were first conducted, suggesting that the maximum concentrations measured in the river were always correlated with low flow conditions. In Yuen Long Creek, the highest concentration of chloroxylenol was 140 μg/L on January 15, 2022 (Saturday), which may be correlated to the fifth wave of COVID-19 in Hong Kong. For the two sampling campaigns in Yuen Long Creek (January 12 to 22, 2022 and April 13 to 21, 2022), all risk quotient (RQ) values obtained were higher than 1, while the maximum RQ value was over 25, which strongly proved that chloroxylenol is a potential stressor in Yuen Long Creek and possess a high ecological risk to aquatic organisms. Proper river management and regulation are necessary to improve river water quality and protect aquatic organisms. Four typical phenolic compounds (phenol, 2,4-dinitrophenol, 2,4,6-trichlorophenol and pentachlorophenol) were not detected in any of the water samples. At last, the removal efficiencies of chloroxylenol were found to be strongly dependent on the technology implemented in the sewage treatment plants (STPs); biological treatment was found to be highly effective in removing chloroxylenol due to microbial degradation. The results of this study could help health and environmental authorities to plan policies, as well as to raise public awareness of the potential ecological consequences of the use of products containing chloroxylenol.

Conventional fingermark detection techniques cannot process large crime scenes and bulky evidence items with large surface areas effectively in a cheap, safe, quick and simple manner. A new and innovative latent fingermark detection technique that uses dry powder from a fire extinguisher was found to detect fingermarks with excellent quality and contrast on nonporous surfaces. The results depicted that fire extinguisher dry powders offered a selective interaction with the moisture and oily components in the fingermark residues, and thus offer clear and sharp images of fingermark ridges, even for latent fingermarks with low deposition quality. Energy dispersive X-ray (EDX) analysis also confirmed the presence of poly(methylhydrosiloxane) (PMHS) with hydrophobic -Si-CH3 groups, as well as the hydrophobic silica micro-particles, which could attribute to the selectivity of fire extinguisher dry powders to fingermark residues. These powders had been proven to outweigh traditional fingerprint powders and cyanoacrylate fuming due to four reasons: (1) cheap and commercially available, (2) safe as particles with average sizes of around 15 μm are large enough to be effectively captured using face masks, (3) high selectivity to fingermarks with weak deposits, such as old fingermarks up to 12 weeks old and (4) quick and simple by spraying the entire crime scene. The results could help forensic scientists to make an informed choice when selecting a detection technique for '*in situ'* latent fingermarks.

To be incorporated into the field of forensic science, the use of fire extinguisher dry powder must be compatible with matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) which is a powerful analytical tool to provide both chemical information and spatial information of endogenous and exogenous chemicals in friction ridges. MALDI-MS protocol for fingermark analysis was first optimized and the best results were achieved in the positive mode when using 4 passes of α-cyano-4-hydroxycinnamic acid (CHCA) at a concentration of 5 mg/mL in 70:30ACN/0.1%TFA solution as matrix. The spraying process of fire extinguisher dry powders to develop the latent fingermarks allows the direct analysis of endogenous compounds (e.g., fatty acids, cholesterol esters, diglycerides, wax esters and triglycerides) and exogenous compounds (due to prior handling of a condom, an aspirin pill or personal and household products) embedded in the fingermarks, as well as the imaging of their distributions without disturbing the fingermark patterns. The simultaneous visualization of latent fingermarks and the recording of a chemical profile of the spatial distribution of the chemical species not only provide valuable evidence about the individual such as his/her lifestyle and recent activities, but also resolve overlapping fingermarks which are commonly found at crime scenes. The feasibility of using MALDI-MSI with fire extinguisher dry powders as the new *in-situ* fingermark development technique was demonstrated. This type of work has never been reported and our results were highly useful to forensic scientists utilizing the full potential of latent fingermarks.

Research publications

Journal papers

1. Mak, S. Y.; Yao, Z. P. Latent fingermark detection using fire extinguisher dry powder as a novel "*in situ*" development powder. (Submitted to journal)

2. Mak, S. Y.; Yao, Z. P. Investigation of chemical analysis on latent fingermarks developed with fire extinguisher dry powder with mass spectrometry imaging (Under preparation)

Conference papers

1. Mak, S. Y. Comparisons of fingerprints development using fire extinguisher dry powder and traditional powders (aluminium powder and magnetic powder). 3rd World Conference and Exhibition on Forensic Science 2019. Germany, July **2019**. (Poster)

2. Mak, S. Y.; Lai, K. W. Latent fingerprint detection using fire extinguisher dry powder on common porous and non-porous surfaces. 4th Global Webinar on Forensic Science. Webinar, March **2022**. (Poster)

3. Mak, S. Y.; Gohel M. D. I. A preliminary investigation into chloroxylenol, a popular antimicrobial ingredient in hygiene and disinfection products, in rivers of Hong Kong. The 10th International Congress of Asian Society of Toxicology. Taiwan, July **2023**. (Poster)

4. Mak, S. Y.; Yao, Z. P. Development of Fire Extinguisher Dry Powders for Analysis of Latent Fingerprints by MALDI-MS. Asia-Oceania Mass Spectrometry Conference and the Annual Meeting of the Korean Society for Mass Spectrometry (AOMSC-KSMS 2023). Korea, August **2023**. (Poster)

5. Mak, S. Y.; Gohel M. D. I. A preliminary investigation into chloroxylenol, a popular antimicrobial ingredient in hygiene and disinfection products, in rivers of Hong Kong. 2023 8th Asia Conference on Environment and Sustainable Development (ACESD 2023). Japan, November **2023**. (Poster)

6. Mak, S. Y.; Yao, Z. P. An investigation into chloroxylenol, a popular antimicrobial ingredient in hygiene and disinfection products, in water environments of Hong Kong. The 21st International Symposium on Toxicity Assessment (ISTA 21). Japan, August **2024**. (Poster)

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At last, I would like to express my deepest gratitude to my family, who have supported and encouraged me throughout this journey in helping me achieve my goals.

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List of abbreviations

Full Form	Abbreviation
2,4-dinitrophenol	2,4-DNP
2,4,6-trichlorophenol	2,4,6-TCP
2,5-dihydroxybenzoic acid	DHB
α-cyano-4-hydroxycinnamic acid	CHCA
adenosine 5'-triphosphate	ATP
assessment factor	AF
chemically enhanced primary treatment	CEPT
Centre for Applied Science and Technology	CAST
Coronavirus Disease-2019	COVID-19
deoxynucleotide acids	DNA
didecyldimethylammonium	DDDMA
diacylglycerol	DAGs
dimethylbenzylammonium	DMA
dimethyldioctadecylammonium	DDA
electrospray ionization	ESI
electrospray ionization mass spectrometry	ESI-MS
energy dispersive X-ray spectroscopy	EDX
Environmental Protection Department	EPD
Fourier Transform Infrared	FTIR
gas chromatography mass spectrometry	GC-MS

high-performance liquid chromatography	HPLC
hydrophilic-lipophilic balance	HLB
indium tin oxide	ITO
liquid chromatography mass spectrometry	LC-MS
mass-to-charge ratio	*m/z*
matrix-assisted laser desorption/ionization mass spectrometry	MALDI-MS
matrix-assisted laser desorption/ionization mass spectrometry imaging	MALDI-MSI
mass spectrometry	MS
measured environmental concentration	MEC
method detection limit	MDL
method quantification limit	MQL
multiple reaction monitoring	MRM
neodymium-doped yttrium aluminium garnet	Nd:YAG
parachlorometaxylenol	PCMX
personal care products	PCPs
pharmaceuticals and personal care products	PPCPs
poly(methylhydrosiloxane)	PMHS
predicted no-effect concentration	PNEC
risk quotient	RQ
severe acute respiratory syndrome coronavirus 2	SARS-CoV-2
scanning electron microscope	SEM

scanning electron microscopy with energy dispersive X-ray spectroscopy	SEM/EDX
sewage treatment plants	STPs
signal to noise ratio	S/N
sinapinic acid	SA
solid phase extraction	SPE
tandem mass spectrometry	MS/MS
thin-layer chromatography	TLC
time-of-flight	TOF
triacylglycerols	TAGS
triclocarban	TCC
triclosan	TCS
ultra-performance liquid chromatography-electrospray	UPLC-ESI/MS/MS
ultra-violet	UV
wax esters	WEs

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Chapter 1: Introduction

1.1 Introduction of mass spectrometry

Mass spectrometry (MS) is a robust and powerful analytical technique that provides both qualitative and quantitative information of a sample by identifying and quantifying various analytes based on their mass to-charge ratios (*m/z*). Because of its ability to structurally characterize organic and inorganic compounds[1], as well as macromolecules such as proteins[2], lipids[3] and deoxynucleotide acids (DNA)[4] in high specificity and sensitivity, MS is applicable across a wide range of scientific fields, such as environmental analysis, pharmacokinetics, drugs analysis, metabolomics, proteomics and forensic analysis.[5]

A mass spectrometer includes three main components: ion source, mass analyzer and detector. The atoms and molecules of interest are first introduced into the ion source of the mass spectrometer, where they are first ionized to form either positive or negative ions.[6] Depending on the ionization technique and polarity mode being used, ions can be seen as molecular ions (protonated or deprotonated) or as ion adducts (sodiated or potassiated). Next, the ions break apart into smaller fragments and are separated according to the *m/z* of the ionized analytes in the mass analyzer.[7] Finally, the detector quantifies the ions, and a mass spectrum is produced, showing graphically the abundance of the ion on the y-axis and the *m/z* on the x-axis.[6]

Many different MS methods and instruments such as ion sources, mass analyzers and detectors have been developed.[8-9] Over the last decade, soft ionization techniques are introduced and extensively used to ionize samples of thermally labile large supramolecules (i.e., proteins, DNA, carbohydrates, lipids and polymers) with small amounts of energy.[10-11] They can cause minimal to no fragmentation and allow the molecular ion to remain largely intact in the ion source, thus easier to identify compounds and allow fast comparison between samples.[10-11] Examples of soft ionization techniques are chemical ionization, fast atom bombardment, desorption electrospray ionization, laser desorption ionization, electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). The quadrupole, ion trap, time-of-flight, orbitrap and various combinations are commonly used modern mass analyzers. With dramatic instrument improvements in offering exceptional capabilities in terms of sensitivity, specificity, speed and automated computer data acquisition, MS has emerged as a powerful and versatile analytical tool to obtain quantitative, structural and compositional information of various organic compounds and large macromolecules.[1-5]

1.2 Electrospray ionization

Electrospray ionization (ESI) is a soft ionization technique in which ions are ejected from charged vapor droplets by applying a high voltage and they repel each other forming a fine plume of ions

upon entering the mass spectrometer for analysis. It was developed in 1989 by Fenn to address the limitations of fast atom bombardment in ionizing large molecules at high yields.[10] ESI can produce multiple charge ions, which is different from other ionization methods.[10, 12]

The transfer of an analyte from a solution into the gas phase by ESI involves three major steps: (1) formation of a fine spray of charge droplets, followed by (2) solvent evaporation of the droplets and (3) formation of gas-phase ions.[12-13] Figure 1-1 shows the schematic representation of the process.[12-13] A sample solution is introduced to the high-voltage capillary by a mechanical syringe pump at low flow rate of typically 1-20 μL/min.[10] Under the high-voltage electric field of 10^6 V/m, the accumulated charges are repelled, allowing the charged droplets of the same polarity as the capillary voltage to drift towards the liquid surface at the capillary tip. Charges at the surface are destabilized under a very high electric field, causing the meniscus to be drawn out and deformed into a Taylor cone at the end of the capillary that contains an excess of charge.[14-15] With the assistance of high temperature and nitrogen drying gas, the solvent in the charged droplets is continuously evaporated, leading to an increase of surface charge density and a decrease of the droplet size to nm level. Finally, when the repulsive force between the charges reaches the Rayleigh charge limit, which is sufficient to overcome the surface tension of the droplets, ions at the surface of the droplets are kinetically and energetically enough to be ejected into the gaseous

phase to form smaller charged droplets.[14] The emitted charged analyte ions are then accelerated into the mass analyzer for subsequent analysis.

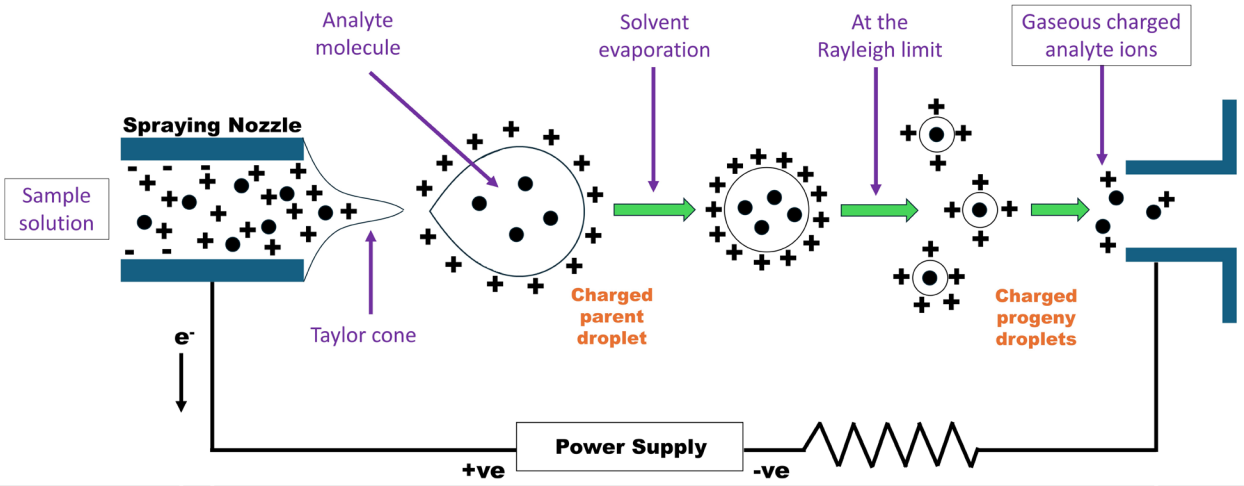


Figure 1-1. Schematic representation of the three major steps in ESI.[12-13]

Electrospray ionization mass spectrometry (ESI-MS) coupled with high-performance liquid chromatography (HPLC) is a sensitive, robust and reliable technique that separates compounds by their physicochemical properties in a highly complex mixture, followed by the detection and measurement of femto-mole quantities of the analytes of interest.[16-18] The molecules can be non-volatile and thermally labile which cannot be readily analyzed by other conventional techniques. With the additional separation capabilities of tandem mass spectrometry (known as MS/MS) and the automated sample introduction, analysis of trace organic compounds such as environmental pollutants in complex environmental matrices can be performed rapidly with high sample throughput.[19-21]

1.3 Matrix-assisted laser desorption/ionization

MALDI is one of the most widely used ionization techniques as it offers more advantages than other techniques: soft ionization, high sample throughput, ability to image with high spatial resolution (5-300 μm), minimal sample preparation, salt tolerant and label-free.[22-23] It uses a laser to ionize and vaporize a small amount of sample that is co-crystalized with a laser energy-absorbing matrix[24], which is then drawn into the mass spectrometer for analysis. This soft ionization technique is particularly important for non-volatile, high molecular weight compounds such as peptides[25], proteins[26], oligonucleotides[27] and oligosaccharides[28].

The mechanism of MALDI involves three major steps: (1) analyte/matrix preparation, (2) desorption of the sample and matrix material and followed by (3) ionization of the analyte molecules. Figure 1-2 shows the schematic representation of the process.[29] Various sample preparation methods can be utilized to analyze different analytes and sample types. To achieve homogenous co-crystallization and allow desorption and ionization of the analyte molecules without excessive fragmentation, a suitable laser-absorbing matrix material such as α-cyano-4-hydroxycinnamic acid (CHCA), 2,5-dihydroxybenzoic acid (DHB) and sinapinic acid (SA) is used.[30] A matrix is typically a weak organic acid with a strong absorbance at the wavelength of the laser and can be applied to the sample by spraying, sputtering, spotting or sublimating.[31] Once

the sample has dried, it is placed into the sample compartment of the instrument where it is irradiated with a high energy ultra-violet (UV) laser pulse. Commonly used UV lasers include nitrogen laser and neodymium-doped yttrium aluminium garnet (Nd:YAG) laser, which emit energy at 337 nm and 355 nm respectively.[32] The laser energy causes analyte-matrix crystals to be vibrationally excited and triggers ablation and desorption, creating a hot gaseous plume of the sample and matrix molecules.[24] Since the excess matrix absorbs most of the pulsed laser energy, the matrix molecules are more readily ionized.[24] In the expanding plume of ablated gases, they collide with the analyte molecules which are ionized by simple proton transfer to be protonated or deprotonated.[24] The ionized analytes are then accelerated into the mass analyzer, typically time-of-flight (TOF), in which ions are separated by their *m/z* according to the time to travel through a field-free flight tube located between the ion source and the detector. One of the limitations of MALDI is the high background noise derived by the matrix, especially in the low *m/z* range.[33] As the choice of matrix and the matrix deposition method are crucial in obtaining reproducible mass spectra, instrument parameters and sample/matrix preparation conditions should be optimized before analyzing the analytes of interest in samples.[34]

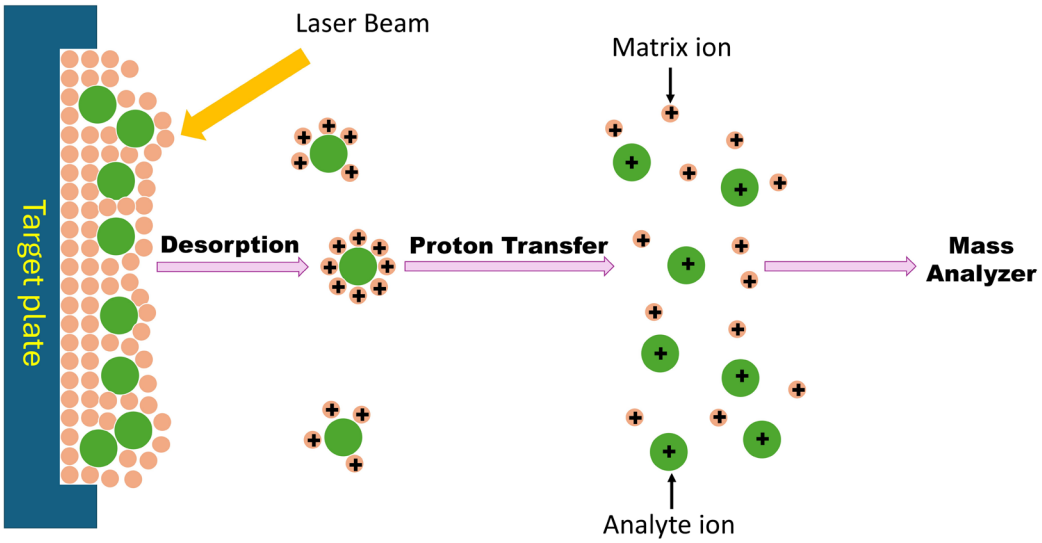


Figure 1-2. Schematic representation of the MALDI ionization process.[29]

Matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI) has been employed as a robust non-destructive imaging technology for *in situ* analysis of a broad range of molecules (e.g., proteins, peptides, drugs and their metabolites as well as pharmaceutical components, endogenous cell metabolites, lipids and other analytes) simultaneously on tissues samples with high sensitivity in one single analysis.[35-40] Since the first paper on MALDI-MSI being published in 1997,[41] the technique has an enormous impact on various life science fields including medicine, pharmaceuticals, biotechnology and microbiology. MALDI-MSI uses laser ionization to raster across a tissue slice collecting mass spectra pixel by pixel, with a spatial resolution ranging from approximately 200 µm down to 20 µm by adjusting the size and spacing of laser pulses.[34] A mass spectrum is obtained for each measuring spot, giving qualitative and quantitative information of different molecules.

1.4 Outline of this thesis

This research study focuses on the application of MS in the field of environmental science and forensic science. We aimed to understand and address the environmental challenges to protect the natural world, as well as to create new and novel protocols for the collection and analysis of latent fingermarks at crime scenes with the needs of criminal investigations.

In **Chapter 1**, the background of MS was introduced. As this research study focused on soft ionization techniques such as ESI and MALDI, the basic working principles and applications of ESI-MS and MALDI-MS were discussed.

In **Chapter 2**, chloroxylenol as an antimicrobial ingredient was first introduced, bringing out the objectives of the research study. SPE followed by analysis via UPLC-ESI/MS/MS was used to determine the concentrations of chloroxylenol in the water environments of Hong Kong. The analytical performances such as method detection limit (MDL), method quantification limit (MQL), precision and accuracy were discussed. Water samples were collected from two rivers and two sewage treatment plants in Hong Kong. Temporal (diurnal, weekly and seasonal) variations in concentrations and ecological risks of chloroxylenol were investigated. The removal efficiencies of chloroxylenol in the two sewage treatment plants were also discussed.

In **Chapter 3**, fire extinguisher dry powder as a fingerprint development powder was first introduced, bringing out the objectives of the research study. Particle sizes, morphology and elemental composition of different brands of fire extinguisher dry powder and traditional fingerprint powders were first characterized. Selectivity, sensitivity and stability of fire extinguisher dry powder in detecting latent fingermarks were then compared with those of the current fingermark development methods using traditional fingerprint powders and cyanoacrylate fuming in an in-depth and systematic way.

In **Chapter 4,** the importance of MALDI-MSI in fingermark analysis was first introduced, bringing out the objectives of the research study. An optimized method for profiling and imaging of endogenous and exogenous compounds on latent fingermarks that have been developed with fire extinguisher dry powder were discussed. The compatibility of fire extinguisher dry powder with chemical imaging of latent fingermarks was investigated.

In **Chapter 5,** a concise summary of the main findings was given. The significance and the unique contributions of the research study made to the fields were explained, bringing out the conclusions and the prospects.

Chapter 2: Investigation of Chloroxylenol in Water Environments of Hong Kong by Mass Spectrometry

2.1 Introduction

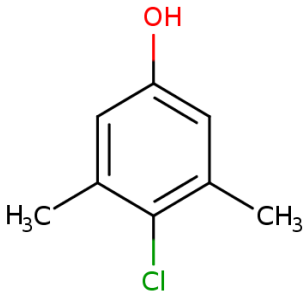
In recent decades, pharmaceuticals and personal care products (PPCPs) has gained widespread public attention as emerging contaminants due to their detrimental effects to the ecological environment and human health.[42-44] As Hong Kong is one of the most densely populated cities with rapid population growth and economic development, PPCPs are extensively and increasingly used to prevent or treat diseases, as well as to improve the quality of daily life.[44-45] This causes PPCPs as well as their metabolites and transformation products to be released continuously into the water environments (surface water, groundwater, wastewater, seawater and drinking water) through diverse routes, including hospital discharges, improper disposal, agricultural runoffs, industrial services, production sites of PPCPs and sewage treatment plants (STPs).[46-49] To date, more than hundreds of pharmaceuticals such as antibiotics and anti-inflammatory drugs, and compounds in fragrances, sunscreen agents and disinfectants/antiseptics have been detected in surface water and wastewater with concentrations ranging from ng/L to μg/L.[42-49] Despite the low concentrations detected, their characteristic behaviors such as persistence, bioaccumulation and toxicity are found to have negative effects on aquatic organisms at chronic exposure, including endocrine disruption, reproductive disorder, genotoxicity, carcinogenicity and fetal development.[50-54]

Since the 1960s, popular broad-spectrum antibacterial agents, such as triclosan (TCS; 5-chloro-2-(2,4-dichloro-phenoxy)-phenol) and triclocarban (TCC; N-(4-chlorophenyl)-N-(3,4-dichlorophenyl) urea), have been widely used in personal care products (PCPs), including hand disinfecting soaps, kitchen detergents, shampoos, shower gels, hand sanitizers, medical disinfectants and cosmetics.[55-56] The chronic exposure of these two compounds were reported to be associated with a variety of harmful health endpoints, including endocrine disruption, decreased fecundity and body development, increased risk of asthma and spontaneous abortion rates, and altered activity of endogenous hormones.[57-59] Due to their underlying potential human health impact and ecotoxicity to aquatic organisms, countries such as the United States have already banned the use of TCS and TCC in over-the-counter antiseptic wash products.[60] To adapt to laws or regulations, there are many enterprises trying to find surrogate antibacterial ingredients to replace the banned TCS and TCC.

Chloroxylenol, a halogenated phenolic derivative commonly known as parachlorometaxylenol (PCMX), is an active antiseptic and antimicrobial ingredient of various over-the-counter-products, such as antibacterial hand sanitizers and soaps, wound-cleansing applications and household disinfectants, which can apply to living tissues and non-living surfaces to kill microorganisms.[61] It is also frequently used in commercial, hospital and industrial settings to control bacteria, algae, fungi and virus. While the exact mechanism of action of chloroxylenol against common infectious

germs remains unknown, it is suggested that the hydroxyl (-OH groups) of the compound and cytoplasmic membrane proteins in the cell membrane of microorganisms have interactions, disrupting the proton gradient of the cell membrane necessary for bacteria to produce adenosine 5'-triphosphate (ATP), which retards the passage of nutrients, resulting in starvation, a loss of normal enzyme activity, cell disruption and rapid cell death.[62-63] It also changes the permeability of the cell walls of microorganisms and hinders their biological processes.[64] Information of chloroxylenol is shown in Table 2-1.

Table 2-1. Information of chloroxylenol.

Name of compound	Chloroxylenol
Chemical name	4-chloro-3,5-dimethylphenol
Abbreviation	PCMX
CAS number	88-04-0
Molecular formula	C_8H_9ClO
Molar mass	156.61 g/mol
Structural formula	
Uses	- An antimicrobial compound applied in hospitals and households for disinfection and sanitation - An active ingredient in many top-selling antibacterial hand sanitizers and soaps, cleaning solutions and household hygiene disinfectants from popular brands such as Dettol, Walch, Watsons and Ariel - Effective against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes COVID-19

COVID-19 has affected over 775 million people globally as of 28 April 2024,[64] and has caused great changes in our daily habits and lifestyle behaviors. For example, the use of hand sanitizers increased by 1.4 to 3 times during the first year of the outbreak.[65] After chloroxylenol was first reported to have good virucidal efficacy against SARS-CoV-2 and emerging mutational variants in May 2020,[66] the use of over-the-counter-products (e.g., antibacterial hand sanitizer, hand wash, disinfectant spray, personal care wipes, liquid and bar soap) and household disinfectants (e.g., antiseptic liquid, surface wipes, surface cleanser and laundry sanitizer) in which chloroxylenol is the main active antimicrobial ingredient has drastically increased worldwide.[67-68]

In Hong Kong, chloroxylenol can be found as an active ingredient in various over-the-counter antibacterial hand sanitizers, cleaning solutions and household hygiene disinfectants from top-selling brands such as Dettol, Walch, Watsons and Ariel. Table 2-2 shows examples of household disinfectants from each brand. Low concentration of chloroxylenol from 0.1 to 0.4% is also used as preservative in shampoos and cosmetic products such as creams. Dettol, being one of the most popular brands in disinfection products, experienced a significant increase in global sales during the pandemic,[67] and has also been ranked first in the sales value in the hand wash segment of liquid soap category for ten years in Hong Kong.[68]

Table 2-2. Examples of popular household disinfectants in Hong Kong.

Brand	Dettol	Walch	Watsons	Ariel
Examples of household disinfectant	Antiseptic liquid 	Multiple-purpose disinfectant 	Antibacterial disinfectant 	Mite removal laundry sanitizer 
Percentage of chloroxylenol	4.8%	4.5%	4.8%	Not indicated

Chloroxylenol is expected to dominate the commercial market as it is added to 56.3% of household disinfectants and 33.9% of hand sanitizers in China.[69] In Hong Kong, there is also an increasing trend of using chloroxylenol as an antibacterial ingredient. However, the use of chloroxylenol is unmonitored and unregulated. It is not included in the routine river water quality monitoring programme implemented by the Government. Labelling of chloroxylenol on the consumer products is also not subject to any formal control or guidelines. In 2019, the Consumer Council indicated that only 20% of the antibacterial hand wash products had labelled chloroxylenol as the antibacterial ingredient on the packaging.[70] Therefore, the total number of antibacterial products using chloroxylenol as the active antimicrobial ingredient in the market is unknown. Table 2-3 shows examples of antibacterial hand wash products which have failed to properly label the presence of chloroxylenol.

Table 2-3. Examples of popular antibacterial hand wash products in Hong Kong.[70]

Brand	Examples of antibacterial hand wash products	Percentage of chloroxylenol
Mannings	Protect Clean Max Protect Anti-bacterial Hand Wash	0.49%
Walch	Anti-bacterial Foaming Hand Wash - Refreshing	0.17%
Walch	Anti-bacterial Hand Wash - Moisturizing	0.17%
Dettol	Original Anti-bacterial pH-balanced Hand Wash	0.16%
Select	Anti-bacterial Hand Wash (Pine Scented) - Refreshing	0.15%

Due to the extensive use of antibacterial hand sanitizers and household hygiene disinfectants in homes and public settings during the pandemic, large amounts of chloroxylenol would be released continuously into the water environments (surface water, groundwater, wastewater, seawater and drinking water) through diverse routes, including hospital discharges, improper disposal from unsewered villages, agricultural runoffs, various sized industrial or commercial processes, production sites of PCPs and inefficient removal from STPs.[46-49] Environmental Protection Agency in the United States has stated that chloroxylenol is moderately toxic to aquatic invertebrates and highly toxic to freshwater fish.[71] An in-vivo study of rainbow trout indicated that chronic exposure of chloroxylenol at concentrations of 4.20 μg/L would induce mutagenic, genotoxic and histopathological effects, while necrotic hepatocytes in liver and glomerulus degeneration in kidney were also observed.[72] Exposure of chloroxylenol was also associated with hatching delay or inhibition, embryonic mortality, morphological malformations, body curvature, and neurotoxicity in zebrafish.[73]

Chloroxylenol has been detected in various water environments such as surface water and wastewater with concentrations ranging from ng/L to µg/L.[69,74-79] Table 2-4 shows the quantification results of chloroxylenol in some previous studies. The concentration levels varied and were found to be dependent on the geographical locations of sample collection, effectiveness of wastewater treatment, types of water sample, rainfall and seasons.[69,74-79]

Table 2-4. Quantification of chloroxylenol in different regions worldwide.

Year of water collection	Region	Locations	Concentration ranges of chloroxylenol	Mean
2020[74]	Hong Kong	River	0.2 - 10.6 µg/L	2.2 µg/L
		Seawater	0.1 - 0.5 µg/L	0.2 µg/L
		Wastewater	0.1 - 65.6 µg/L	/
2017[69]	Guangzhou, China	Urban streams and Pearl River	1.62 - 9.57 µg/L	/
2017[75]	Tianjin, China	Beitang River	Wet season (ND - 3.7 µg/L) Dry season (ND - 1.36 µg/L)	/
		Dagu River	Wet season (ND - 157 µg/L)	/
2016[76]	Taiwan	Wuluo River	Fall (10 - 23 ng/L) Summer (100 - 200 ng/L)	/
2015[77]	Kuwait, Middle East	Seawater	0.06 - 0.79 µg/L	/
		Effluent outfall	/	43,070 µg/L
2012[78]	Jakarta, Indonesia	Rivers	60 to 1200 ng/L	/
2010[79]	Taihu Lake, China	Surface water	ND - 161.4 ng/L	64.9 ng/L

ND: not detected.

At the same time the investigation of this research study was going on, a research article which studied the occurrence and stability of chloroxylenol in water environments of Hong Kong was published in early 2023.[74] The authors revealed for the first time that chloroxylenol in wastewater is not effectively removed by chemically enhanced primary treatment (CEPT), the most common wastewater treatment processes used in Hong Kong, suggesting that this may cause pollution and contamination to the water environments. Chloroxylenol was detected in 80% of the sampling sites in the rivers and coastal waters of Hong Kong, with concentrations ranging from 0.2 to 10.6 μg/L and 0.1 to 0.5 μg/L, respectively. These concentrations indicated that an immediate threat to marine organisms may not be present, but exposure to chronic levels of chloroxylenol may pose a risk to invertebrates in rivers.[74]

As Hong Kong is one of the most densely populated cities in the world with a widespread reliance on antibacterial hand sanitizers and household disinfectants from well-known brands such as Dettol and Walch, our hypothesis was that the concentrations of chloroxylenol in the water environments of Hong Kong during and after the COVID-19 pandemic might be much higher than those reported in earlier studies. In order to evaluate the harm of chloroxylenol comprehensively, a study on the temporal variations and ecological risk of chloroxylenol in the water environments of Hong Kong is therefore of considerable importance. This can also help raising the public

awareness of the potential ecological consequences from overusing chloroxylenol-based household disinfectants and antibacterial hand sanitizers, and allowing the related authorities to evaluate whether management and control actions are needed. To differentiate from the research article published in early 2023, different rivers were investigated in this research study, while temporal (diurnal, weekly and seasonal) variations in concentrations and ecological risk of chloroxylenol were studied comprehensively to evaluate the harm of chloroxylenol to the water environments of Hong Kong.

In this research study, surface water samples from two rivers in Hong Kong were collected in different days and seasons. The concentrations of chloroxylenol were measured and evaluated to understand the temporal (diurnal, weekly and seasonal) variations and potential ecological risk of chloroxylenol in the river environment of Hong Kong. As there are no treatment facilities or techniques specialized to eliminate chloroxylenol in STPs, discharge of untreated or inadequately treated effluent can cause serious environmental and health problems. Studying the removal efficiencies of chloroxylenol in different STPs can allow us to determine the final output into the water environments and establish a crucial knowledge base that enables health and environmental authorities to strategically plan policies, and systematically evaluate the effectiveness of environmental programs in the community.

Objectives of the research study:

a) To collect surface water samples from two rivers (Tuen Mun River and Yuen Long Creek) in Hong Kong in different days and seasons;

b) To collect influent and effluent wastewater samples from two STPs (Stonecutters Island Sewage Treatment Works and Sha Tin Sewage Treatment Works);

c) To measure the concentration of chloroxylenol in the collected samples using solid phase extraction (SPE) and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS);

d) To analyze the results and study the temporal variations (e.g., diurnal, weekly and seasonal) of chloroxylenol in rivers and to assess the removal efficiencies of chloroxylenol in STPs, and finally, to assess its potential ecological risks to the aquatic ecosystems.

2.2 Experimental section

2.2.1 Chemicals and materials

Chloroxylenol, 4-chlorophenol as the internal standard (IS) and four typical phenolic compounds (phenol, 2,4-dinitrophenol, 2,4,6-trichlorophenol and pentachlorophenol) were purchased from Sigma (St. Louis, USA). They were stored according to the instructions of supplier. HPLC-grade acetonitrile and methanol were purchased from Anaqua Chemicals Supply (Wilmington, USA). Oasis hydrophilic-lipophilic balance (HLB) cartridges (200 mg; 6 mL) purchased from Waters Corporation (Milford, USA) were used in sample clean-up and concentration. 12-port SPE vacuum manifold was purchased from Alltech Associates (Derield, USA). Milli-Q water from a Milli-Q Reference A+ system (Millipore, Billerica, MA) was used when high purity water was required.

2.2.2 Sample collection and storage

Two rivers were the subject of this research study for a period of one year: Tuen Mun River and Yuen Long Creek. They were selected due to their "bad" water qualities reported by Environmental Protection Department (EPD) in 2021.[80] This may be due to the presence of pig and chicken farms, as well as villages without sewerage systems that may allow polluted discharges to reach the aquatic environment. Rivers with better water quality were reported to have lower concentrations of chloroxylenol.[74] Table 2-5 shows the information of the two studied rivers.

Table 2-5. Information of the two studied rivers - Tuen Mun River and Yuen Long Creek.

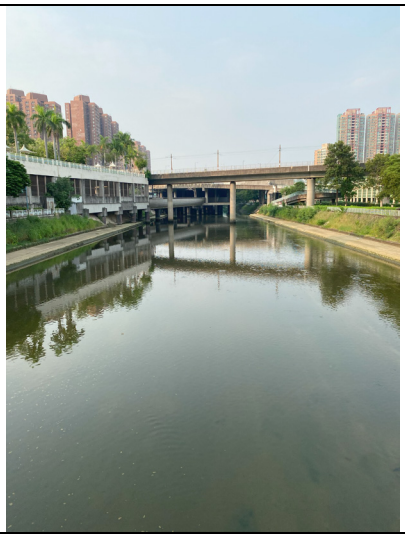
	Tuen Mun River	**Yuen Long Creek**
Description of the river	• Next to Tuen Mun Hospital and residences • Receive and transport domestic sewage and industrial sewage with or without treatment	• A major river in Yuen Long • Next to Long Ping MTR station • Pass through rural areas, the densely populated Yuen Long Kau Hui and new town
Photo of sampling point		
Characteristics	• 38 km long • A catchment area of 16.5 km²	• 60 km long • A catchment area of 27 km²
Population[81]	532 000 in 2023 (7.1% of Hong Kong population)	670,000 in 2023 (9.0% of Hong Kong population)
Water quality graded by the EPD[80]	"Bad" for an upstream monitoring station	"Bad" and "Very Bad" for some downstream stations

Figure 2-1 shows the sampling point selected in Tuen Mun River and Yuen Long Creek, using the river monitoring stations established by the EPD as a reference.[80] A bucket was used to collect the river water samples. Five 200 mL water samples were mixed together, and finally, 1.0 L mixed water as a representative composite sample was stored in an amber bottle which was pre-rinsed with water from each sampling point. Milli-Q water samples were prepared and put aside as field

blanks for quality control. All the collected water samples were transferred to our laboratory in an ice box and were stored at 4º C overnight until being processed for analysis.

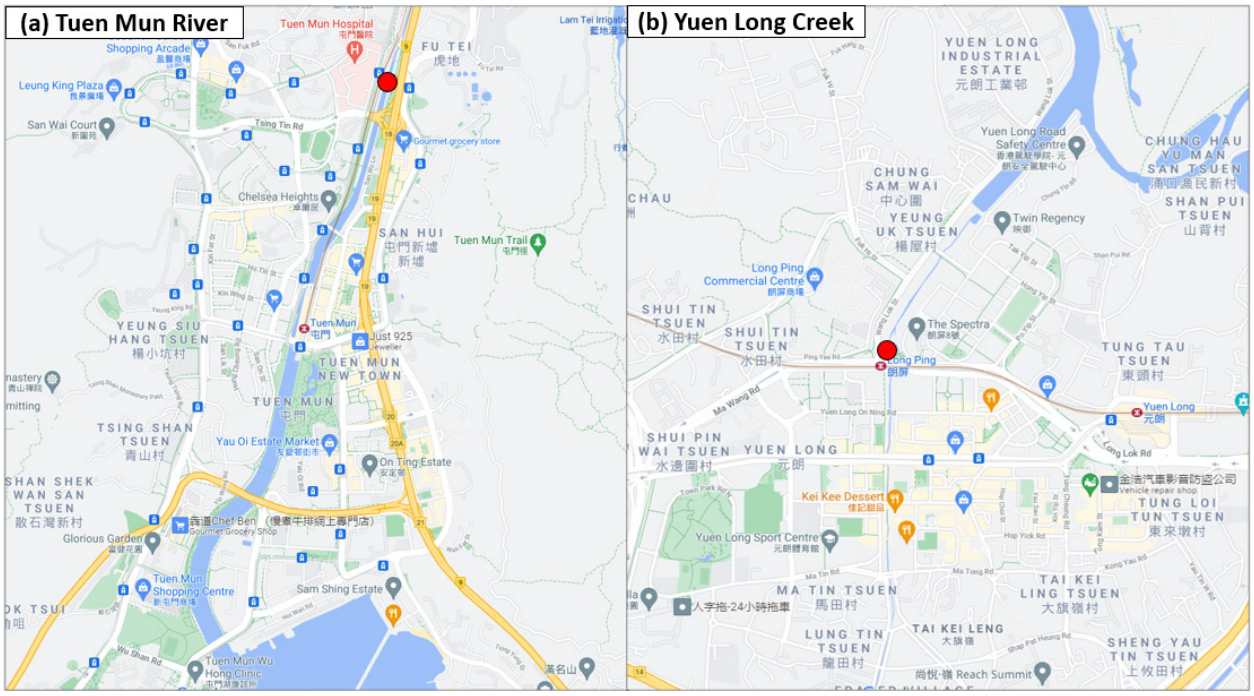


Figure 2-1. Map showing the locations of sampling point in (a) Tuen Mun River and (b) Yuen Long Creek.

In the beginning of the research study, diurnal studies were first conducted by collecting water samples at the sampling point in each river every hour to determine an optimized sampling time. For each sampling day, 12 containers of water samples were collected from 8:00 AM in the morning to 8:00 PM at night. Concentrations of chloroxylenol at each timepoint were measured and a suitable sampling time (i.e., with the highest concentration of chloroxylenol) was determined for the remaining studies. Two sampling campaigns for the diurnal studies were conducted for

each river respectively, i.e., September 12 and October 17, 2021 for Tuen Mun River, and September 26 and November 14, 2021 for Yuen Long Creek. Weekly and seasonal monitoring of chloroxylenol were also performed for a period of one year from July 2021 to July 2022. Each sampling campaign in each river lasted for at least one week.

Influent and effluent wastewater samples were also collected at two STPs with different treatment processes two times during the study, using the same protocol in collecting and preserving river water samples. Table 2-6 shows the information of the two STPs.

Table 2-6. Information of the two studied STPs.

	Sha Tin Sewage Treatment Works	**Stonecutters Island Sewage Treatment Works**
Description	Largest secondary sewage treatment works in Hong Kong	Largest chemically enhanced primary treatment (CEPT) plant in Hong Kong
Serving districts	• Shatin • Ma On Shan	• East Kowloon and Tsuen Wan • Hong Kong Island • Northwest Kowloon
Population serving, capita[81]	690,000 (9% of Hong Kong population)	5,700,000 (80% of Hong Kong population)
Designed flow, m^3/day	340,000	2,450,000
Treatment process	Secondary (biological treatment)	Chemically enhanced primary treatment (CEPT)
Sewage processing time	16 hours	1.5 hour

2.2.3 SPE procedures

SPE extraction followed by LC-MS/MS analysis that can allow accurate and sensitive detection of chloroxylenol in water samples was employed. Sample pretreatment and chemical analysis generally followed the protocol described in the literature.[69] Collected water samples were first vacuum filtered through 0.45 μm glass fibre filter to remove particles. 200 mL of each river water sample was spiked with IS, and SPE was performed with Oasis HLB cartridge using a 12-port SPE vacuum manifold. The cartridge was conditioned consecutively with 6 mL of methanol and 6 mL of Milli-Q water. Water sample was then passed through the preconditioned cartridge connected to a vacuum pump at a flow rate maintained below 5 mL/min. After the cartridge was dried, elution was performed with 6 mL of methanol. The eluate was evaporated, and the residue was redissolved in 1 mL of methanol. After centrifugation at 10000 rpm for 5 min, 600 μL of the supernatant was collected for instrumental analysis.

2.2.4 LC-MS/MS analysis

Chromatographic separation of chloroxylenol was performed on a Waters Acquity UPLC system (Milford, USA) using a CSH C18 column (Waters Corporation; 2.1 × 100 mm, 1.7 μm), which was eluted at a constant volume flow rate of 0.3 mL/min. The injection volume was 5 μL. The mobile phase consisted of two components: water (solvent A) and methanol (solvent B). The

gradient elution program was set as follows: 0.0-7.0 min, 50-75% B; 7.0-9.0 min, 75-100% B; 9.1 min, 50% B; 9.1-10.5 min, 50% B. The UPLC was connected to an QTrap 6500+ MS/MS system (Sciex Instruments, Toronto, Canada), and negative ESI in multiple reaction monitoring (MRM) mode was used. Table 2-7 shows the optimized ESI and MS parameters for maximum analytical sensitivity by injecting a standard solution of chloroxylenol at a concentration of 100 μg/L into the stream of mobile phase.

Table 2-7. Optimal ESI and MS parameters for the quantification of chloroxylenol.

Parameters	Optimal value
Ionization potential	-4,500 V
Gas temperature	550 °C
Curtain gas	25 psi
Gas I	60 psi
Gas II	60 psi

The molecular ion of chloroxylenol at *m/z* 155 was selected and fragmented after applying the collision energy. The fragment ions were monitored, and the collision cell energy and declustering potential were varied to investigate the effect on the fragmentation. The fragment ion and collision cell energy that gave stable and intensive signal were selected for detection and quantification of chloroxylenol. The MRM channels and optimized parameters for the detection and quantitation of chloroxylenol are listed in Table 2-8.

Table 2-8. MRM channels and optimized parameters for the detection and quantitation of chloroxylenol.

Analyte	MRM Channel	Declustering potential (V)	Collision cell energy (V)
Chloroxylenol	155 → 35*	-45	-45
	155 → 119	-23	-23
4-chlorophenol (IS)	127 → 35	-15	-15

*The channel for quantitation of chloroxylenol.

2.2.5 Determination of the concentration of chloroxylenol in water samples

A six-point standard calibration curve was prepared for quantitative analysis by spiking methanol with chloroxylenol and 4-chlorophenol, which was used to normalize the peak area from one sample to another as an IS. The data acquisition and processing of the resultant MRM chromatograms were analyzed using SCIEX OS-Q 2.0 Software (AB SCIEX, Foster City, CA, USA) and Analyst Software (AB SCIEX, Foster City, CA, USA). The calibration curve was constructed by plotting the peak area ratio of chloroxylenol to the IS against the concentration of chloroxylenol in each working standard solution. Three sets of experimental data were averaged. To find out the concentration of chloroxylenol in each collected water sample, the peak area ratios of chloroxylenol and the IS were computed and compared to the corresponding peak area ratio of known chloroxylenol concentration and the IS in the standard calibration curve.

2.2.6 Method validation

Accuracy and precision

Method validation was performed in both Milli-Q water and blank river water samples. Filtered matrices spiked with chloroxylenol of low (1 µg/L), medium (5 µg/L) and high concentrations (20 µg/L) were prepared for the determination of accuracy and precision of the method at different environmental concentration levels. Analysis was conducted for five times respectively. The accuracy was calculated by comparing the concentrations found in the samples spiked before extraction with the nominal concentrations. Interday precision was calculated by analyzing water samples spiked with the same concentrations of chloroxylenol on five different days in a week, while intraday precision was determined by analyzing water samples on the same day. They were then expressed as the relative standard deviation of the concentrations detected in five replicates.

Method detection limit (MDL) and method quantification limit (MQL)

MDL and MQL were measured by comparing signals of blank river water samples spiked with low concentrations of chloroxylenol against the signals of the blank and determining the minimum concentration of chloroxylenol at which the analyte signal was reliably detected or quantified with the help of SCIEX OS-Q 2.0 Software. The blank river water samples spiked with low concentrations of chloroxylenol, as well as the blanks were subjected to all the steps of the

analytical method which included the sample pretreatment, SPE analyte extraction and MS analysis. MDL is set at a signal to noise ratio (S/N) of three, and MQL is set at a S/N of ten. At least nine measurements were obtained for the determination of MDL and MQL.

2.2.7 Calculations of removal efficiency in sewage treatment plants (STPs)

The removal efficiency of chloroxylenol in each STP was calculated using the below equation, and the sewage processing time was also taken into account to collect the effluent samples corresponding to the influent sample which have been treated.

$$\text{Removal efficiency (\%)} = 100\% \times \frac{\text{Concentration (influent)} - \text{Concentration(effluent)}}{\text{Concentration (influent)}}$$

2.2.8 Environmental risk assessment: risk quotient method

The ecological risk of chloroxylenol in each river water and wastewater sample was characterized by risk quotient (RQ) method. RQ is calculated using the below equation by dividing the measured environmental concentration (MEC) by the predicted no-effect concentration (PNEC), which is the concentration with no adverse effects of exposure in an ecosystem.[82-83]

$$\text{RQ} = \frac{\text{MEC}}{\text{PNEC}} \qquad \text{PNEC} = \frac{\text{Toxicity benchmark}}{\text{Assessment factor (AF)}}$$

The concentration of chloroxylenol in each water sample found by LC-MS/MS analysis was used as MEC. For chloroxylenol, the PNEC value is 5.2 μg/L which was reported in the literature and was derived from the toxicity data divided by an assessment factor (AF) of 1000.[84] In order to account for bioaccumulation and avoid hazard underestimation, RQ values exceeding 1.0 indicate that the compound is a potential stressor and possess a high ecological risk to aquatic organisms, while ecological risk levels are low for $RQ < 0.1$ and medium for $0.1 < RQ < 1$.[82-83]

2.3 Results and discussion

2.3.1 Optimization of MS parameters

The molecular ion of chloroxylenol was confirmed by injecting the standard solution of chloroxylenol into the mass spectrometer. Two MRM transitions with the highest response were chosen as the quantitative (Q) and confirmative (C) ions respectively: m/z 155→35 (quantitative transition) and 155→119 (qualitative transition) for chloroxylenol; m/z 127→35 (qualitative transition) for the IS. Figure 2-2 shows the MS/MS spectrum of chloroxylenol in a standard solution.

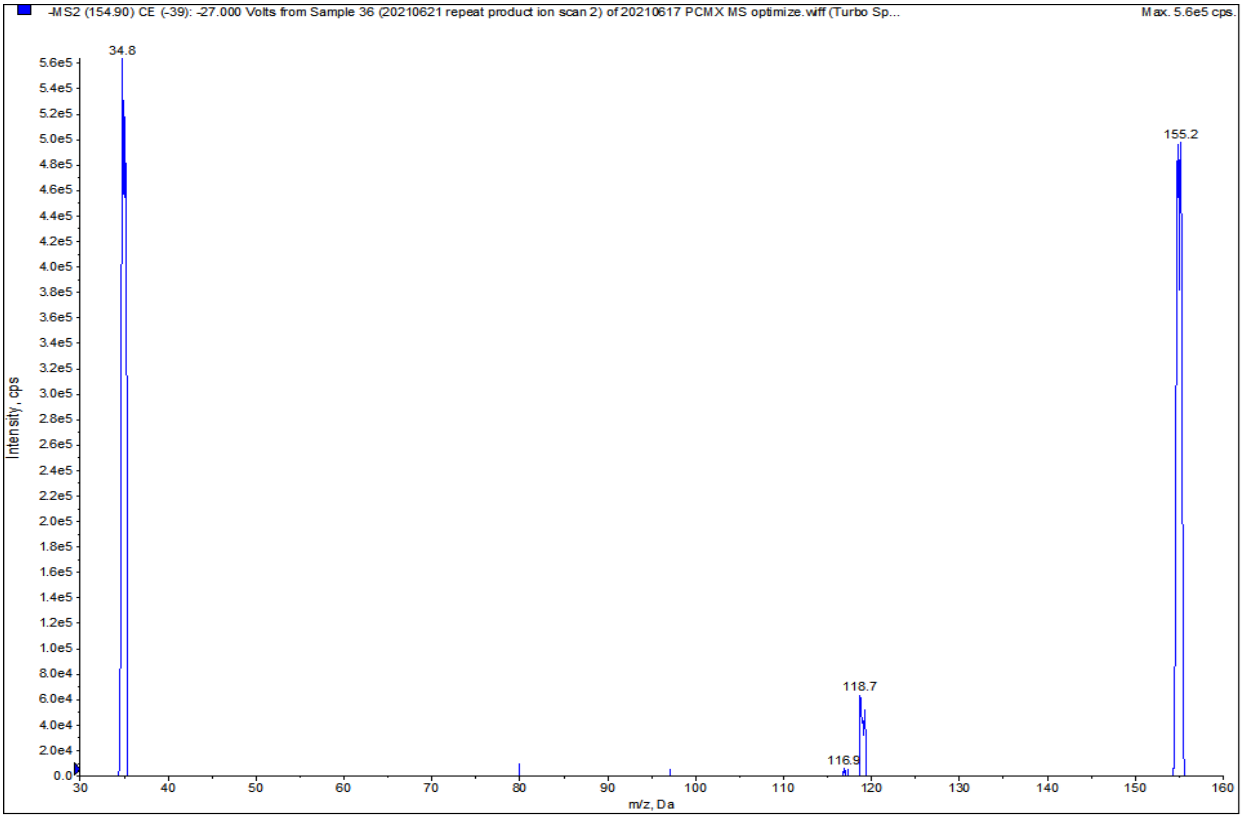


Figure 2-2. MS/MS spectrum showing the molecular ion and fragment ions of chloroxylenol.

Typical MRM chromatograms for the detection of chloroxylenol in different concentrations of standard solutions are shown in Figure 2-3 and 2-4. Each standard solution was spiked with 500 µg/L IS and the time required for each sample to complete the run was around 10 minutes. Sharp peaks with strong ion signals were observed for all standard solutions with different concentrations of chloroxylenol, while the peak area for IS, which was at fixed concentration of 500 µg/L, did not change significantly between different standard solutions.

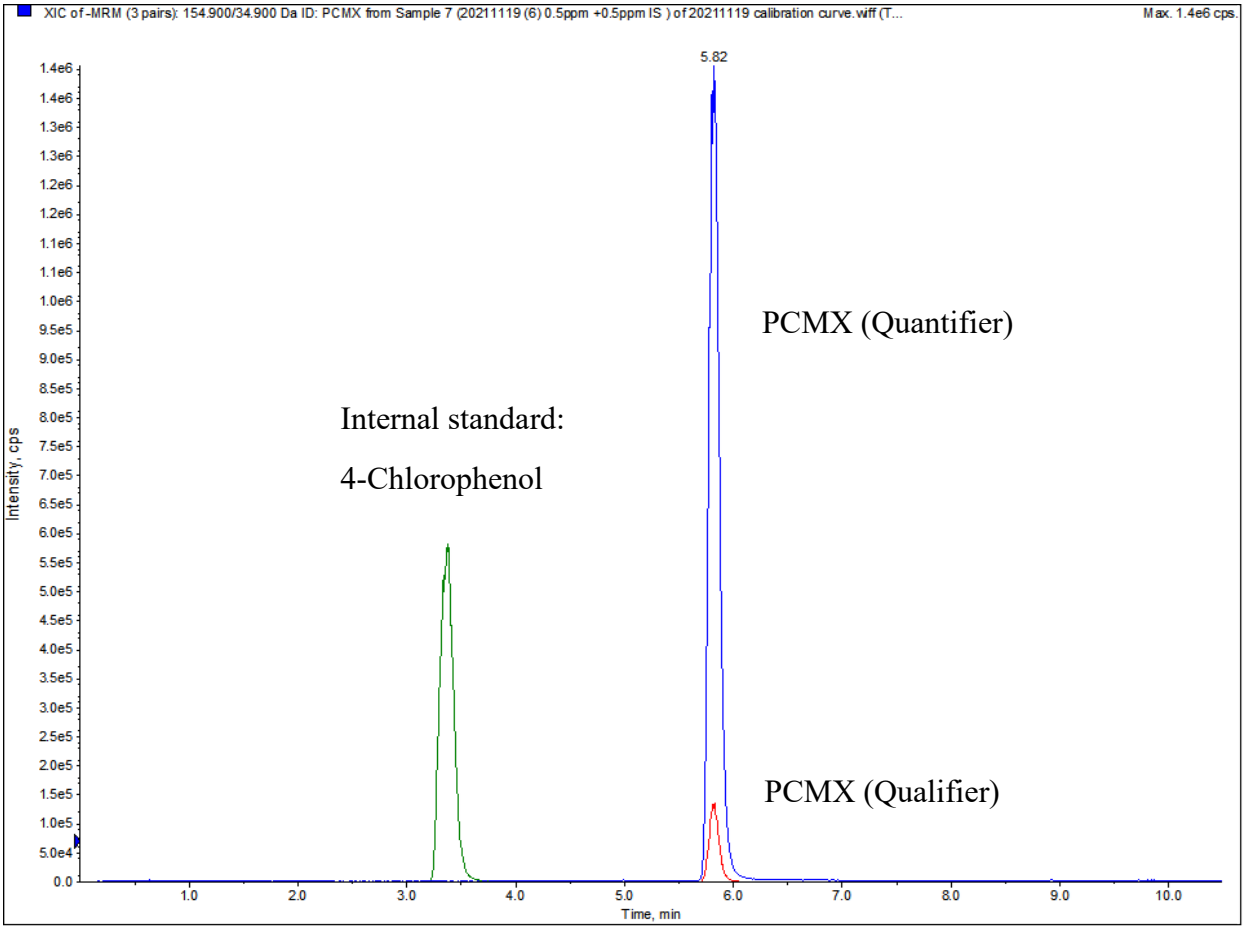


Figure 2-3. Typical MRM chromatogram of a standard solution with 500 µg/L chloroxylenol and 500 µg/L IS.

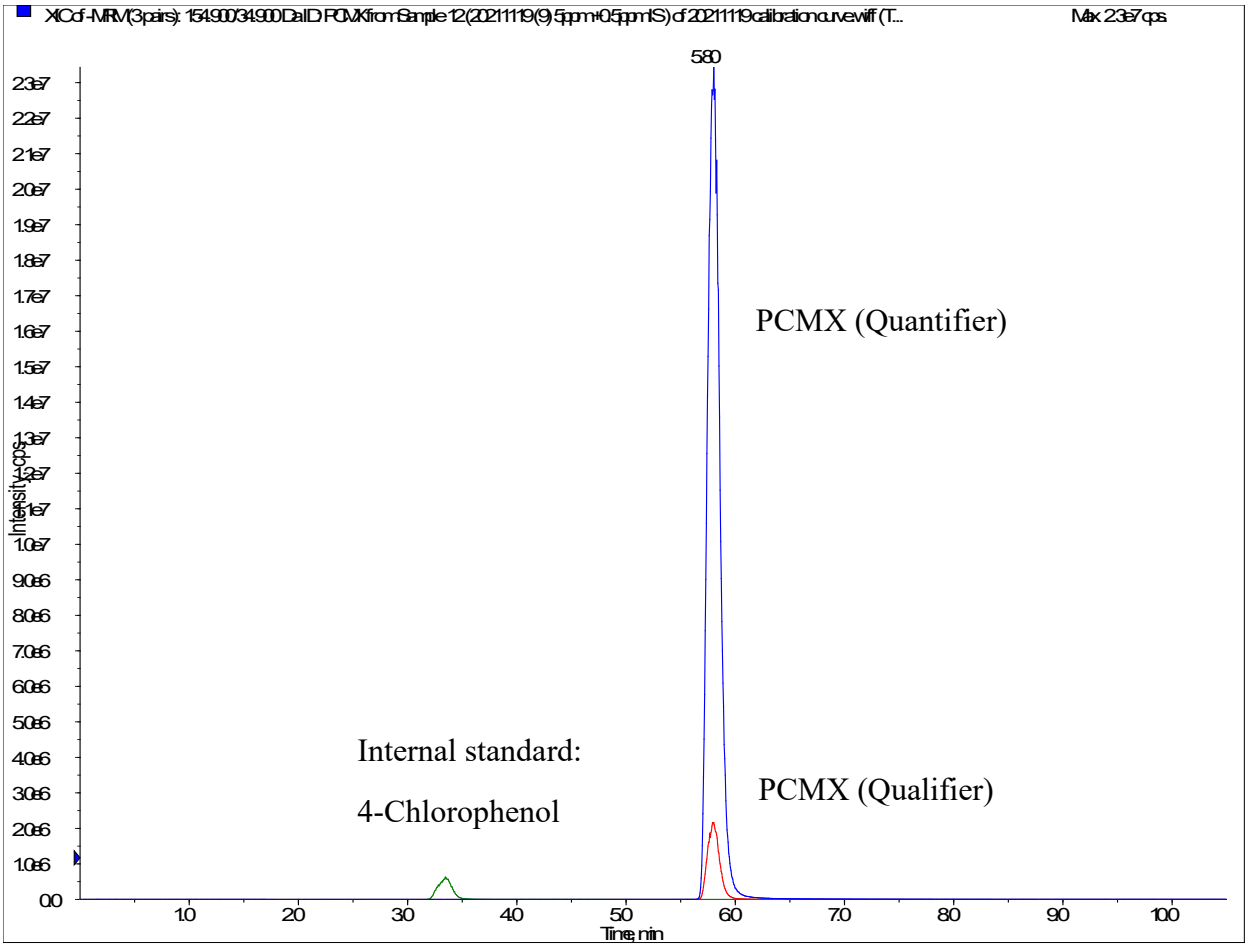


Figure 2-4. Typical MRM chromatogram of a standard solution with 5,000 µg/L chloroxylenol and 500 µg/L IS.

The calibration curves for the quantitation of chloroxylenol were constructed by measuring the peak area of at least six spiked samples with different concentrations of chloroxylenol. A typical calibration curve obtained for quantitation of chloroxylenol is shown in Figure 2-5. It shows a calibration curve covering a concentration range of 0.001-10,000 µg/L with linearity of R^2 = 0.9964. The small error bars indicate the high reproducibility of preparing the standard solutions and constructing the calibration curve. The peak area of MRM signals for chloroxylenol exhibited

a positive correlation with the increase in the concentrations of chloroxylenol, while the peak area for IS, which was at fixed concentration of 500 µg/L, did not change significantly between different standard solutions. Construction of a calibration curve with six data points could be completed within one hour.

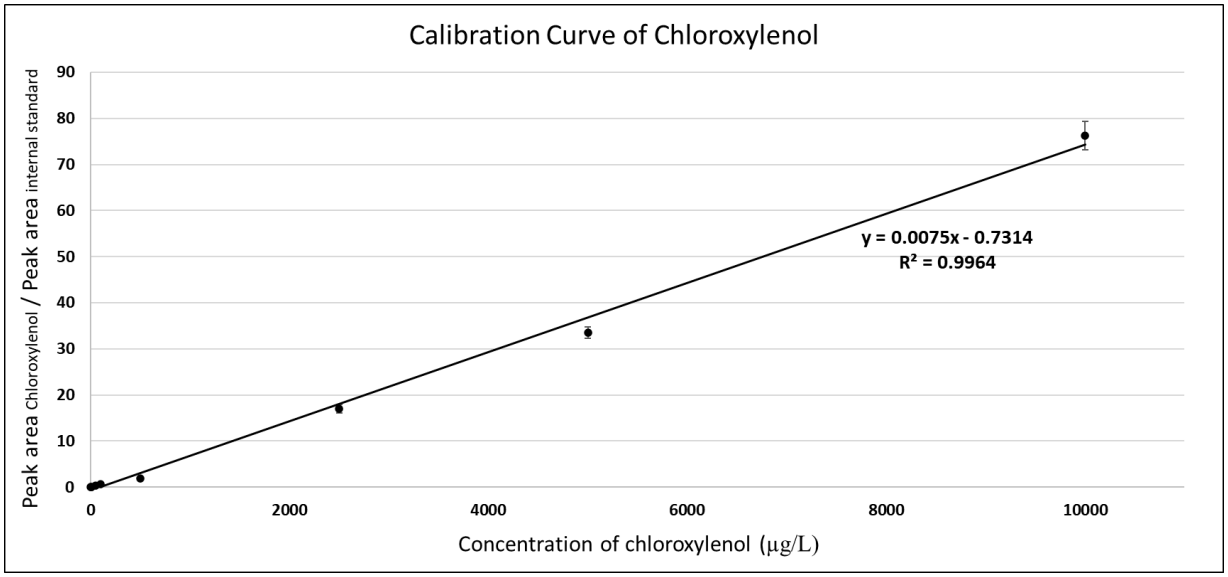


Figure 2-5. A calibration curve obtained for quantification of chloroxylenol.

2.3.2 Method validation

Figure 2-6 shows a typical MRM chromatogram for the detection and quantification of chloroxylenol in river water samples. Although high complex matrix composition was present in the river water samples, similar to the standard solutions, the peak area for IS, which was at fixed concentration of 500 µg/L, did not change significantly between different river water samples.

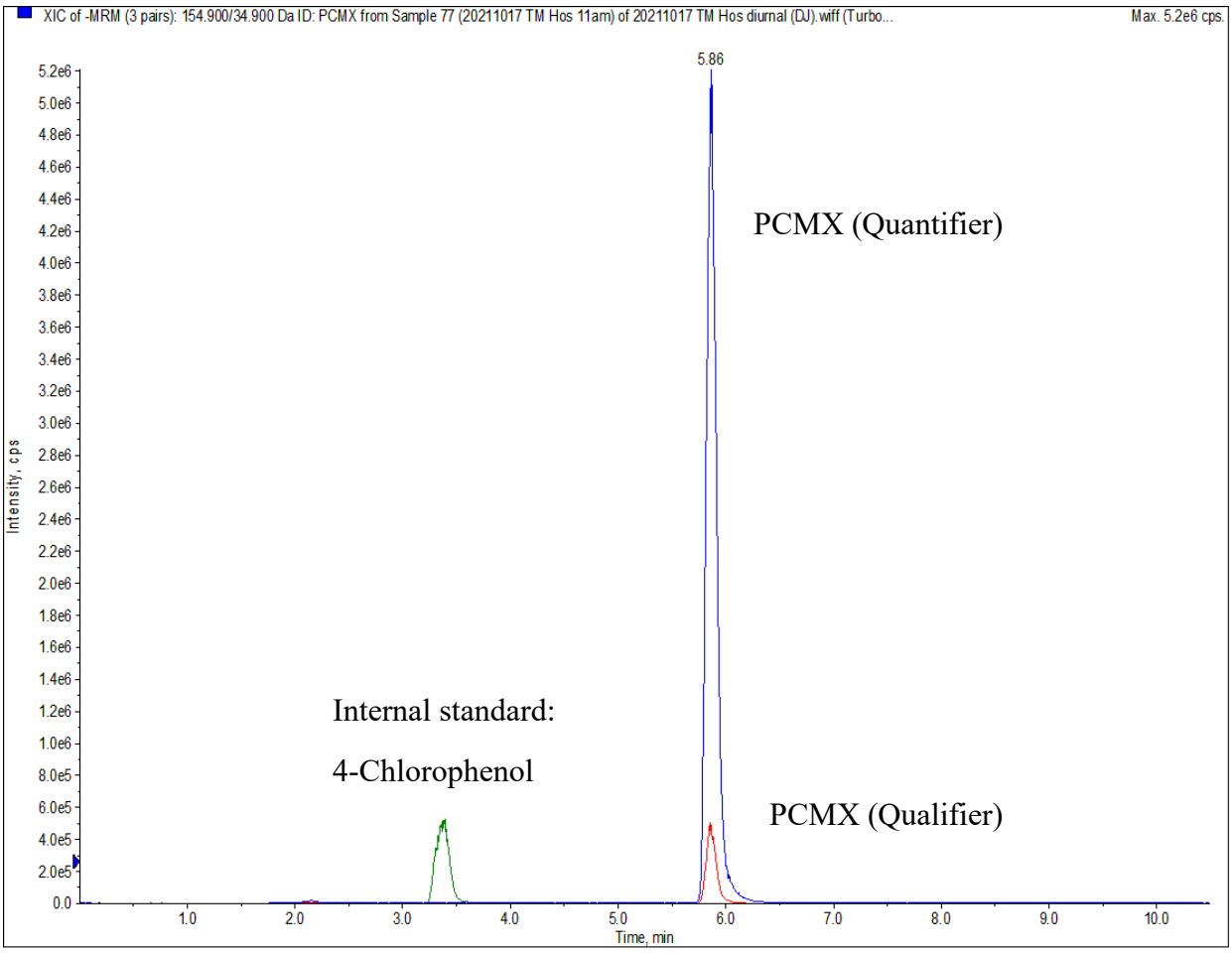


Figure 2-6. Typical MRM chromatogram of river water samples collected in Tuen Mun River.

Accuracy and precision of quantitative analysis of chloroxylenol

Spiked samples at low (1 µg/L), medium (5 µg/L) and high environmental concentrations (20 µg/L) of chloroxylenol in river water were tested. The accuracy and precisions of the quantitative analysis results are summarized in Table 2-9. The accuracy was in the range of 80-95%, which was very close to the requirement of 80-120%, while both intraday and interday precisions were

found to be under 10%, indicating that the present SPE analyte extraction and MS method had acceptable and satisfactory accuracy and precision for the quantitation of chloroxylenol.

Table 2-9. Accuracy and precision of SPE and LC-MS/MS method for the quantification of chloroxylenol in river water samples.

	Spiked quantity (µg/L)	Determined quantity ± S.D. (µg/L) (n=5)	Accuracy (%)	RSD (%)
River water	1	0.9 ± 0.1	89.7	17.7
	5	4.3 ± 0.4	86.5	10.0
	20	18.3 ± 1.6	91.3	8.5

MDL and MQL

MQL was found to be 5.0 ng/L giving signals with S/N ≥ 10 as shown in Figure 2-7, while Figure 2-8 indicates the LOD of chloroxylenol to be 2.5 ng/L, which was determined as the concentration of chloroxylenol that could generate signals with S/N ≥ 3. From the river water samples collected, all the concentrations of chloroxylenol detected were at least 0.1 µg/L, indicating that the present analyte extraction and MS method had acceptable MQL for the quantitation of chloroxylenol.

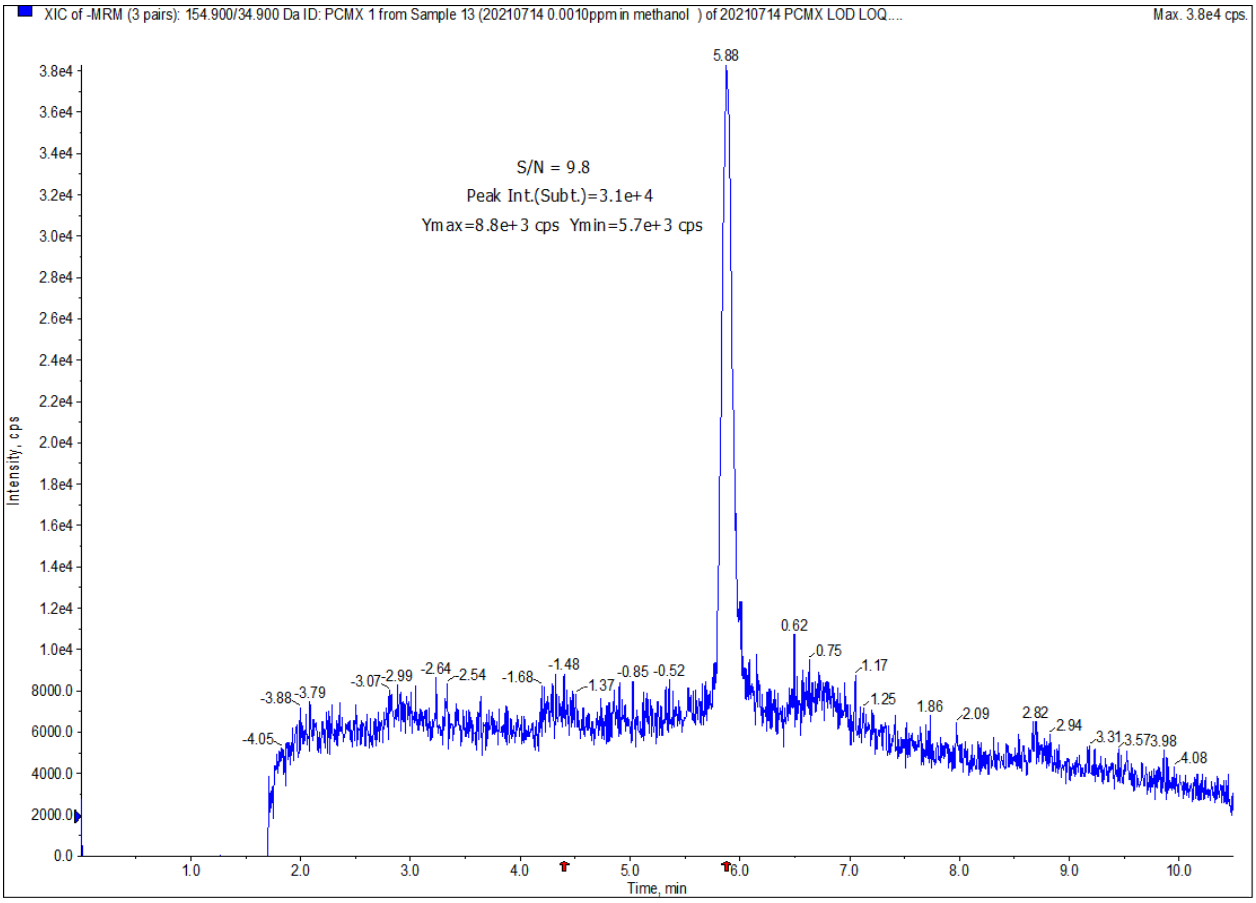


Figure 2-7. MQL of chloroxylenol in river water samples.

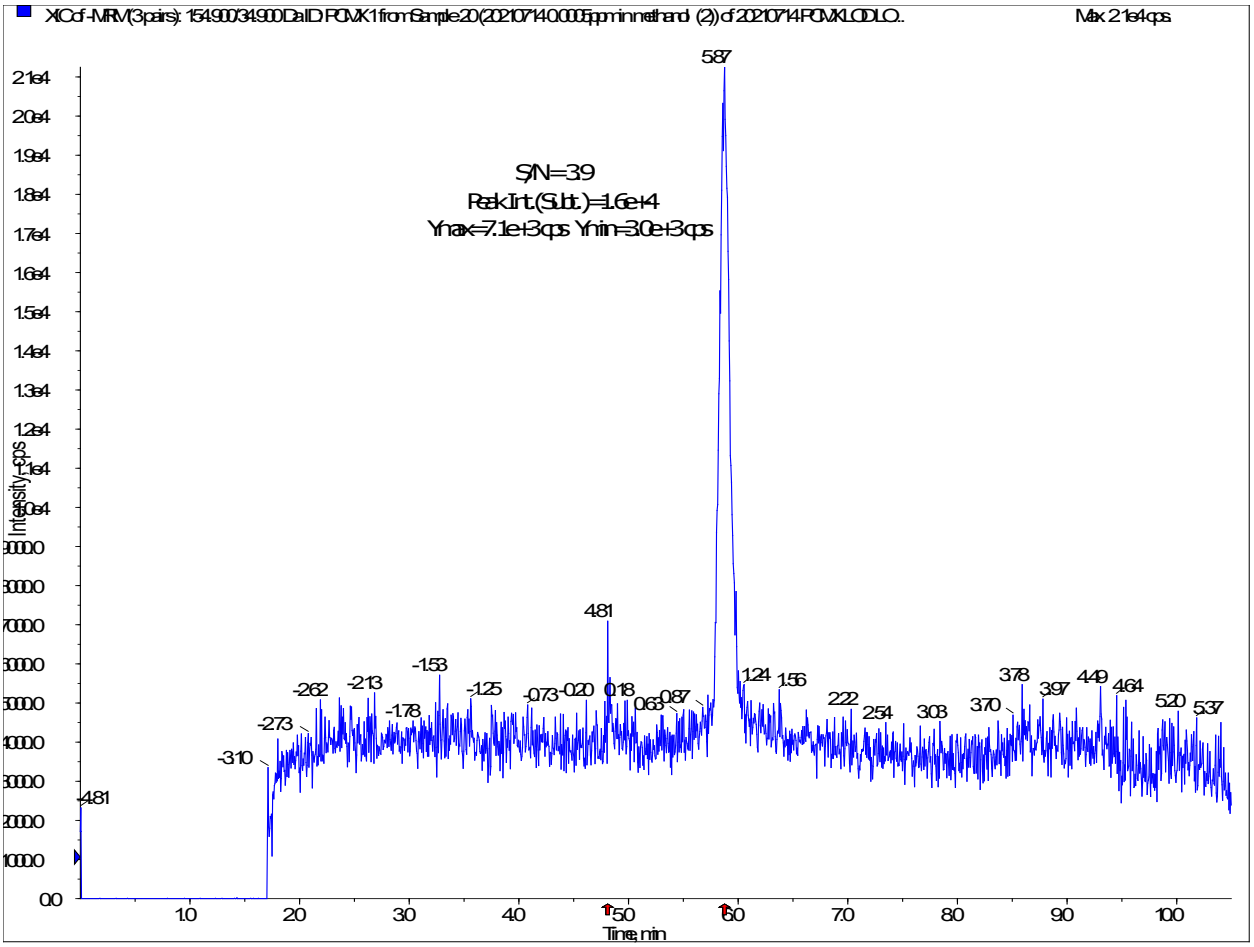


Figure 2-8. MDL of chloroxylenol in river water samples.

2.3.3 Occurrence and concentrations of chloroxylenol in rivers of Hong Kong

Diurnal variations in the concentrations

In the beginning of the research study, diurnal studies were first conducted by collecting water samples at a sampling point in each river every hour. For each sampling day, 12 containers of water samples were collected from 8:00 AM in the morning to 8:00 PM at night. Concentrations of chloroxylenol at each timepoint were then measured. The purpose was to determine a suitable sampling time (i.e., with the highest concentration of chloroxylenol) for the remaining studies.

Two sampling campaigns for the diurnal studies were conducted for each river respectively, i.e., September 12 and October 17, 2021 for Tuen Mun River, and September 26 and November 14, 2021 for Yuen Long Creek. Figure 2-9 shows the photographs taken at the sampling point in Tuen Mun River at different timepoints on September 12, 2021. Apparent changes in the differences of water levels could be seen at high tides and low tides. For each sampling campaign, the tidal status of each day was collected from Tides 4 Fishing (https://tides4fishing.com/), which is based on astronomical tidal height predictions, providing data on the height of the water level and the times they occur. Figure 2-10 shows the photographs taken during sample collection and SPE.

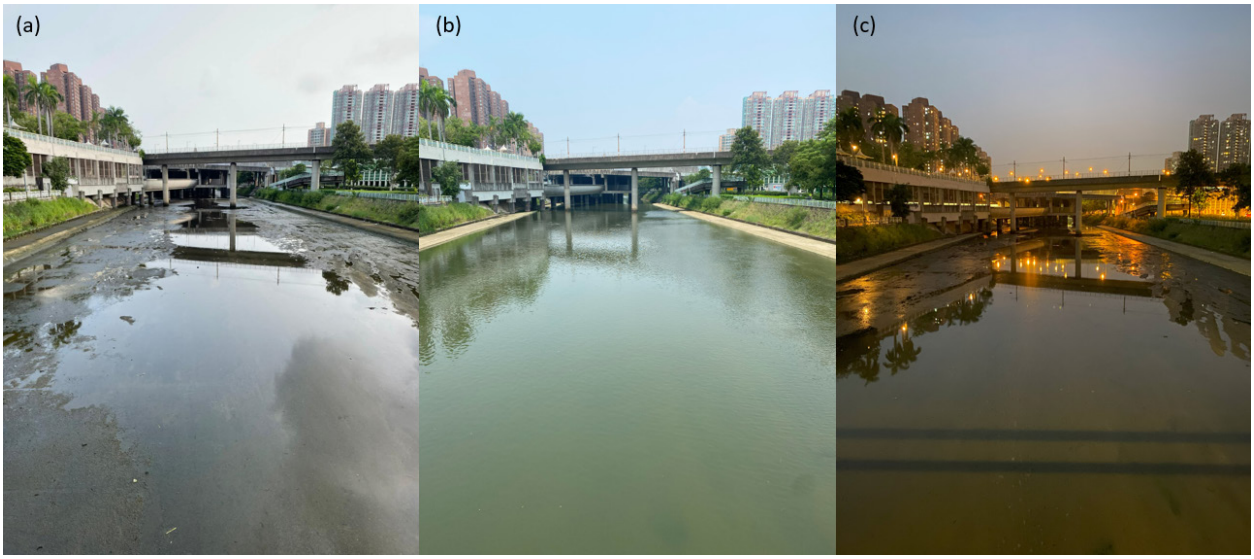


Figure 2-9. Photographs taken at the sampling point in Tuen Mun River at (a) 8:00 AM (first low tide), (b) 1:00 PM (high tide) and (c) 7:00 PM (second low tide) on September 12, 2021.

Figure 2-10. Photos obtained in (a) Tuen Mun River; water sample collected; corresponding glass fibre filter after filtration (from left to right) and (b) Yuen Long Creek (beside Long Ping MTR station); water sample collected; corresponding glass fibre filter after filtration (from left to right).

For each sampling campaign in Tuen Mun River, the tidal status of the day in terms of the height of the water level and the concentration of chloroxylenol at each sampling timepoint was investigated. In the first sampling campaign (September 12, 2021) and the second sampling campaign (October 17, 2021), all river water samples showed the presence of chloroxylenol, with concentrations ranged from 0.19 to 1.66 µg/L and 1.01 to 10.31 µg/L respectively (Figure 2-11).

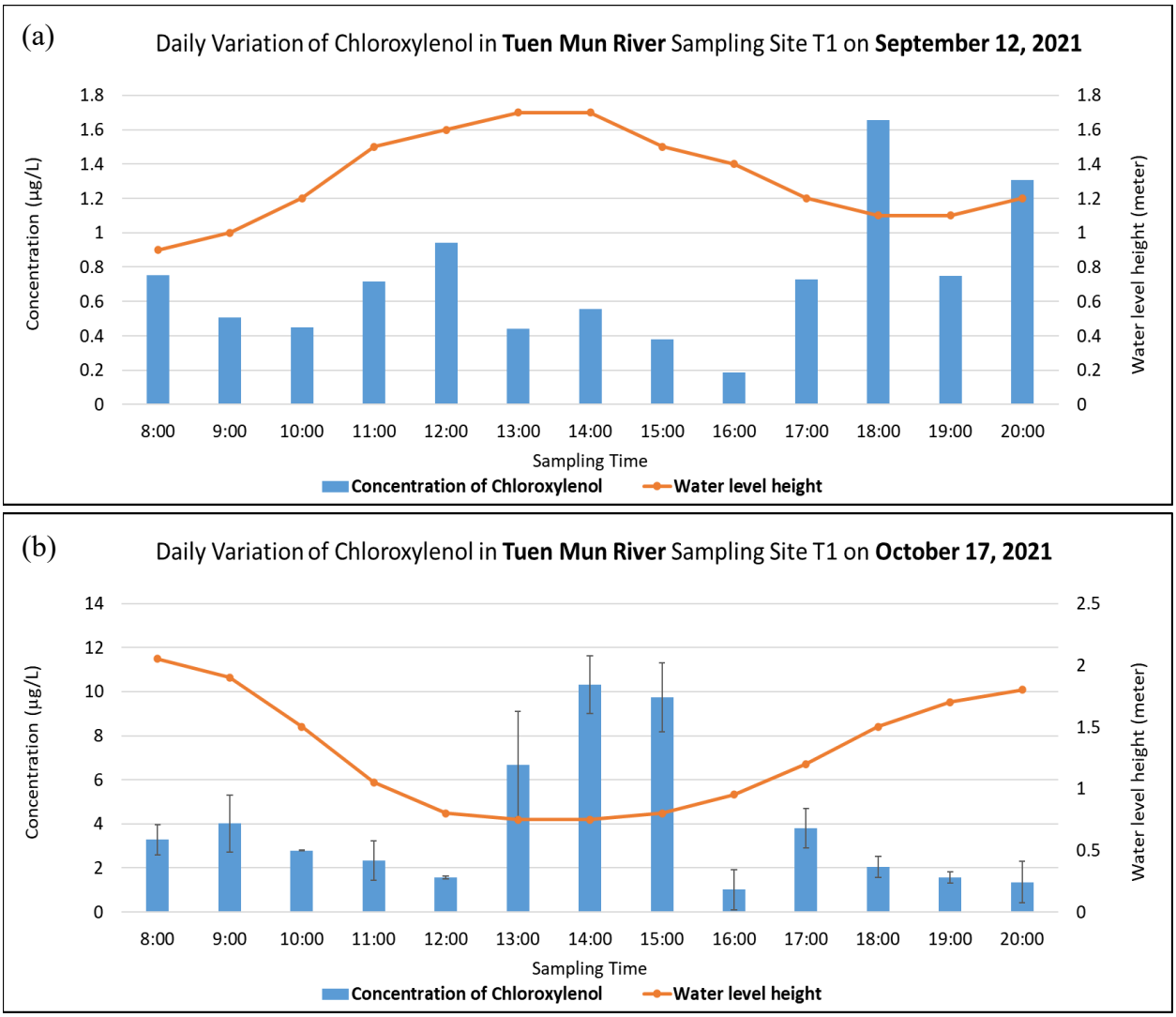


Figure 2-11. Graphs showing the daily variations in the concentrations of chloroxylenol in Tuen Mun River on (a) September 12 and (b) October 17, 2021.

For each sampling campaign in Yuen Long Creek, the tidal status of the day in terms of the height of water level and the concentration of chloroxylenol at each sampling timepoint was investigated. On the first sampling campaign (September 26, 2021) and the second sampling campaign (November 14, 2021), all river water samples showed the presence of chloroxylenol, with concentrations ranged from 1.41 to 5.95 µg/L and 8.33 to 40.60 µg/L respectively (Figure 2-12).

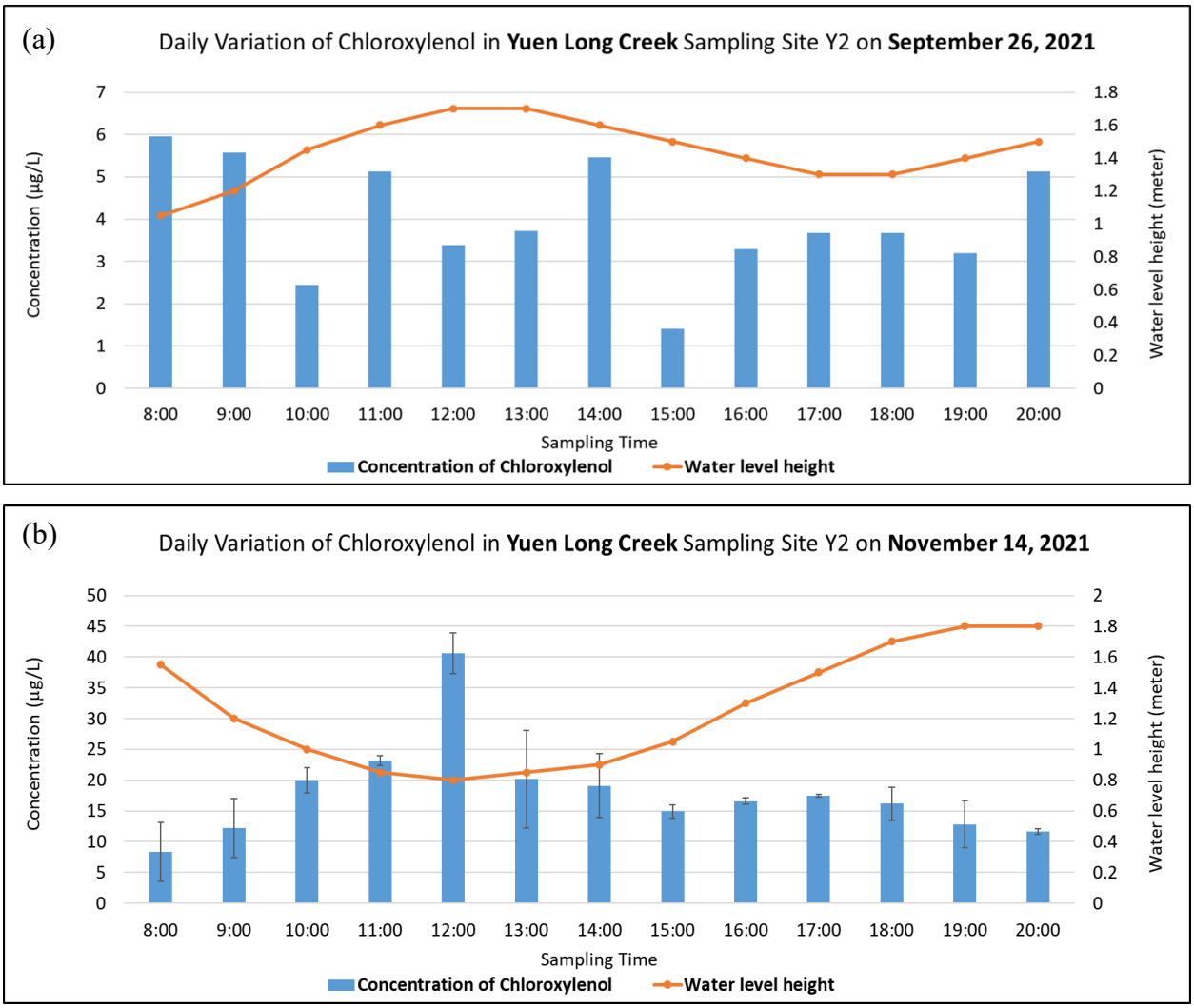


Figure 2-12. Graphs showing the daily variations in the concentrations of chloroxylenol in Yuen Long Creek on (a) September 26 and (b) November 14, 2022.

Figure 2-11 and Figure 2-12 show that the concentrations of chloroxylenol increased during low flow condition and decreased during high flow condition of a river, while the maximum concentration of analyte measured in a river was always correlated with low flow condition, i.e., the river's flow volume had decreased, and the quantity of water was low. The association between concentrations and the tidal status of each day agreed with those reported in previous studies.[49,85]

As it is not feasible to measure the flow of the rivers on-site with a water flow probe or meter, Tides 4 Fishing (https://tides4fishing.com/) was accessed to find out the approximate height of water level and the times they occur. The values of the height of water level were not crucial as our ultimate goal was to determine a suitable sampling time (i.e., with the highest concentration of chloroxylenol) for the remaining studies, and whether sampling should be conducted at high tides or low tides.

Taking September 13, 2021 as an example, there were two low tides from 8:00 AM in the morning to 8:00 PM at night in Tuen Mun River. The first low tide was at 8:07 and the second low tide was at 18:55. Two low tides were indicated in Figure 2-13, and there may be differences in the concentrations of chloroxylenol being measured. As a result, further investigation on the concentrations in the river water samples collected at the two low tides was required.

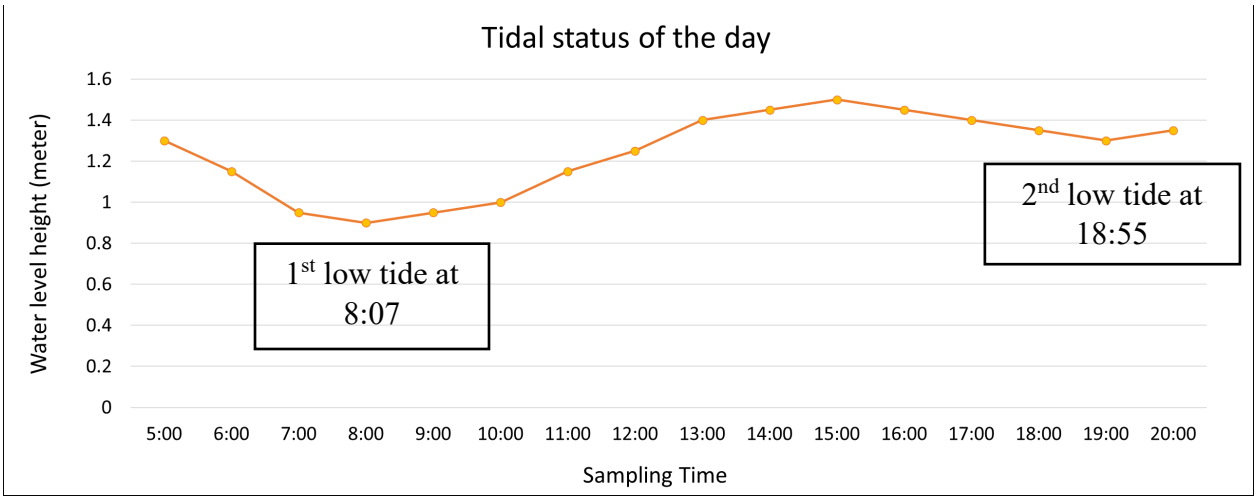


Figure 2-13. Graph showing the height of water level in Tuen Mun River on September 13, 2021.

To investigate the differences in the concentrations of chloroxylenol at the two low tides, a sampling campaign was taken place in Yuen Long between January 19 to 22, 2022. River water samples were collected at the two low tides and concentrations of chloroxylenol were determined. A comparison of the concentrations of chloroxylenol between the first low tide and the second low tide from January 19 to 22, 2022 is shown in Figure 2-14. It was noted that there was a significant difference in the concentrations of chloroxylenol between the two low tides; the concentrations of chloroxylenol at the second low tide was much higher due to domestic human activities, indicating that for the remaining studies, if the extent of contamination was studied, the sampling should be conducted at a sampling timepoint when the second low tide was taken place instead of the first low tide.

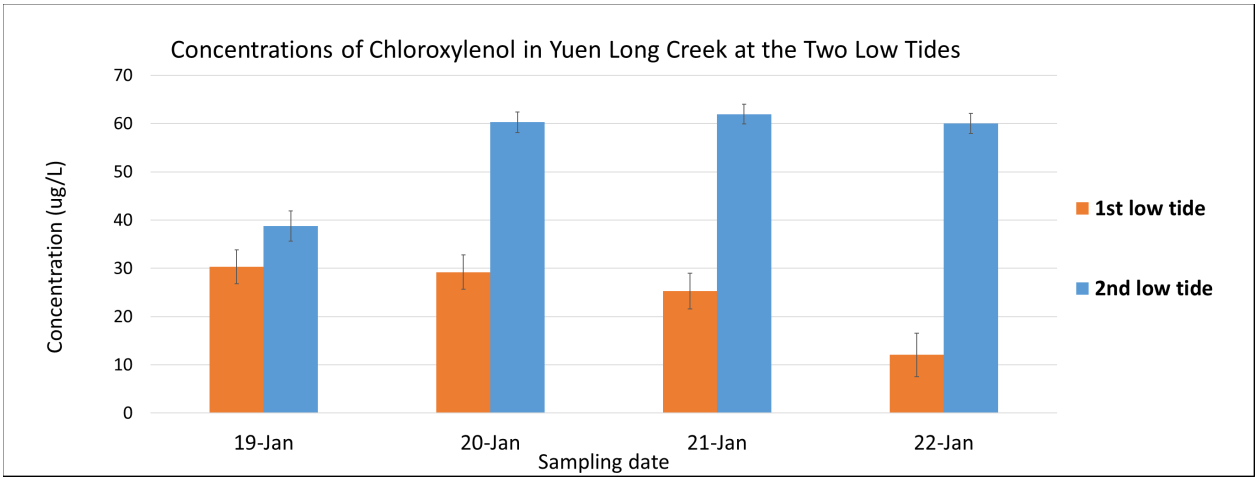


Figure 2-14. Comparison of the concentrations of chloroxylenol between the first low tide and the second low tide in Yuen Long Creek from January 19 to 22, 2022.

Temporal (weekly and seasonal) variations in the concentrations

Weekly and seasonal variations in the concentrations of chloroxylenol in Tuen Mun River and Yuen Long Creek were also investigated from July 2021 to July 2022. Two sampling campaigns were conducted for each river respectively and each sampling campaign lasted for more than one week. All water samples were collected at the timepoint when low tide was taken place (or the second low tide if two low tides could be found between 8:00 AM in the morning and 8:00 PM at night).

In Tuen Mun River, the first sampling campaign which was taken place from November 6 to 20, 2021 showed that the highest concentration of chloroxylenol was measured on November 13 (Saturday). A trend was not observed for the second sampling campaign that was taken place from April 13 to 21, 2022, suggesting that the use of disinfectants and detergents containing chloroxylenol was relatively constant throughout the week. Figure 2-15 shows the weekly variations in the concentrations of chloroxylenol in Tuen Mun River between November 6 to 20, 2021 and April 13 to 21, 2022, respectively.

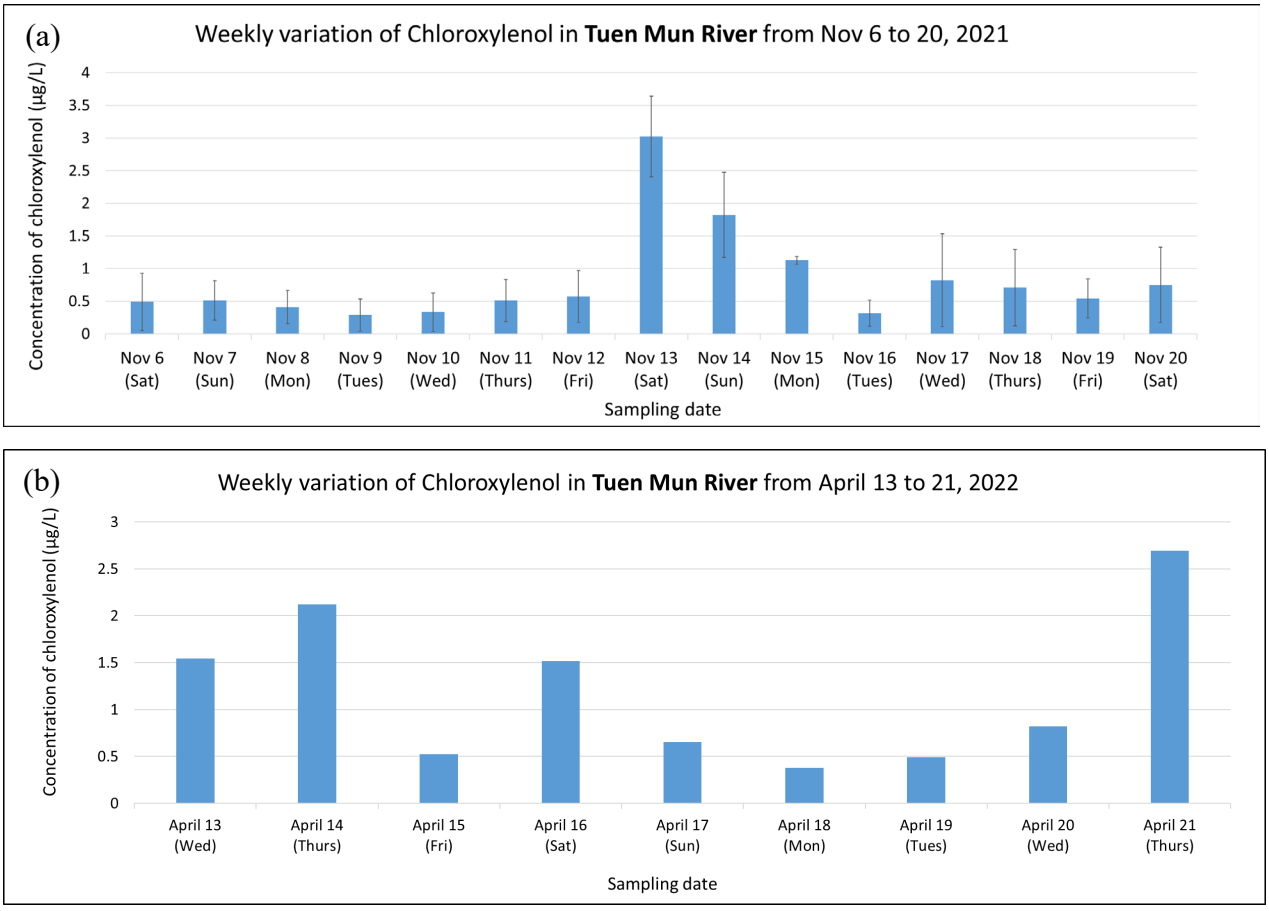


Figure 2-15. Weekly variations in the concentrations of chloroxylenol in Tuen Mun River between (a) November 6 to 20, 2021 and (b) April 13 to 21, 2022.

In Yuen Long Creek, the first sampling campaign which was taken place from January 12 to 22, 2022 indicated the highest concentration of chloroxylenol to be measured on January 15 (Saturday). The highest concentration was found to be 140 µg/L, which was correlated to the fifth wave of COVID-19 in Hong Kong. However, for the second sampling campaign which was taken place from April 13 to 21, 2022, the lowest concentration of chloroxylenol was measured on April 16 which was a Saturday. Figure 2-16 shows the weekly variations in the concentrations of chloroxylenol in Yuen Long Creek between January 12 to 22, 2022 and April 13 to 21, 2022, respectively.

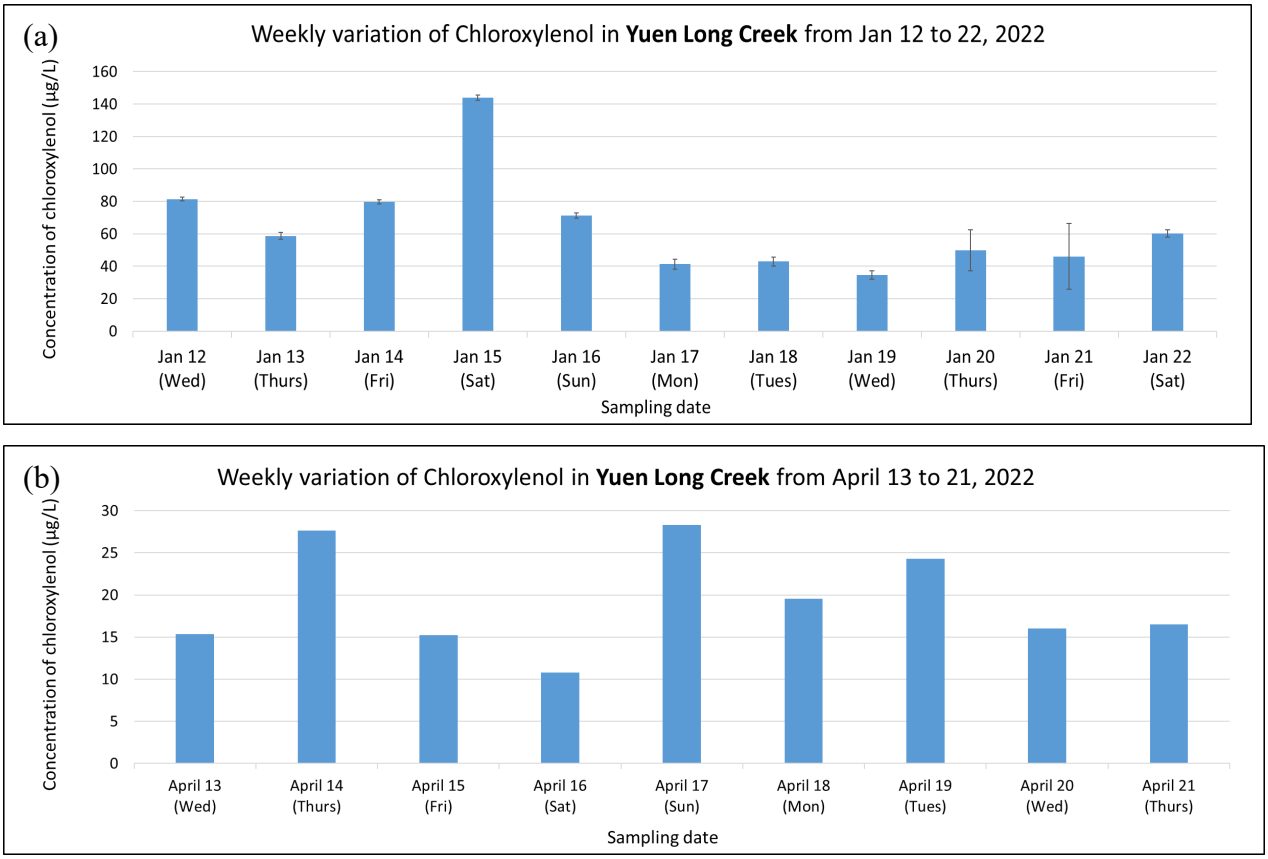


Figure 2-16. Weekly variations in the concentrations of chloroxylenol in Yuen Long Creek between (a) January 12 to 22 and (b) April 13 to 21, 2022.

Water samples were collected in different seasons and all the concentrations of chloroxylenol are shown in Table 2-10. Highlighted in blue, the fifth wave of COVID-19 in Hong Kong started in December 2021, and this may be the reason of the surprisingly high concentrations of chloroxylenol in water samples collected in Yuen Long Creek in January 2022. Although dilutions resulting from rainfall may decrease the concentrations of chloroxylenol being detected, collection of river water samples was postponed for at least three consecutive days when there was no rainfall during that period. Overall, the concentrations of chloroxylenol were not seasonally dependent, assuming that the use of disinfectants and hand sanitizers are relatively constant as the detection frequencies were 100% throughout the year. Furthermore, Yuen Long Creek passes through rural areas, the densely populated Yuen Long Kau Hui and new town. Incomplete coverage of wastewater infrastructure in the rural villages as well as misconnections to stormwater drains may allow chloroxylenol to be released continuously to the river.

Table 2-10. Seasonal variations in the concentrations of chloroxylenol in Tuen Mun River and Yuen Long Creek.

		Tuen Mun River						**Yuen Long Creek**					
	Month	July 2021	Sept 2021	Oct 2021	Nov 2021	April 2022	July 2022	July 2021	Sept 2021	Nov 2021	Jan 2022	April 2022	June 2022
µg/L	**Range**	2.7 - 33.4	0.2 - 1.7	1.0 - 10.3	0.3 - 3.0	0.4 - 2.7	0.1 - 10.2	12.8 - 18.8	1.4 - 6.0	8.3 - 40.6	34.7 - 144.0	10.8 - 28.3	1.8 - 16.4
	Average in each campaign	18.1	0.7	3.9	0.8	1.2	4.5	16.6	4.0	17.9	64.5	19.3	8.2
	Average in each season	18.1	2.3		0.8	1.2	4.5	16.6	4.0	41.2		19.3	8.2

Potential ecological risk of chloroxylenol in rivers of Hong Kong

By looking at the concentrations of chloroxylenol detected in both rivers, we can conclude that Yuen Long Creek was heavily polluted with chloroxylenol as concentrations of chloroxylenol in all of the collected water samples were much higher than 4.20 μg/L (i.e., a concentration causing chronic effects in freshwater fish, involving genotoxicity and histopathology), suggesting that high chloroxylenol concentrations in Yuen Long Creek pose a potential risk of aquatic toxicity. In addition, the chemical combination of chloroxylenol and other compounds may exhibit additive or synergistic toxic effects even the concentrations of chloroxylenol were below 4.20 μg/L in Tuen Mun River.

The ecological risk of chloroxylenol in each river water could also be characterized by RQ method. For the two sampling campaigns in Tuen Mun River (November 6 to 20, 2021 and April 13 to 21, 2022), all RQ values obtained were lower than 1. Over half of the sampling dates had RQ values between 0.1 and 1, indicating that the concentrations of chloroxylenol in Tuen Mun River posed a medium high ecological risk to aquatic organisms. For the two sampling campaigns in Yuen Long Creek (January 12 to 22, 2022 and April 13 to 21, 2022), all RQ values obtained were higher than 1, while the maximum RQ value was over 25, which strongly proves that chloroxylenol is a potential stressor in Yuen Long Creek and possess a high ecological risk to aquatic organisms.

2.3.4 Occurrence and concentrations of other phenolic compounds in rivers of Hong Kong

Four typical phenolic compounds were selected as representative phenolic compounds since they are priority pollutants by exhibiting carcinogenic, neurotoxic, teratogenic and mutagenic properties with adverse effects on aquatic biota and human health.[50-54] The molecular ion of each phenolic compound was selected and fragmented after applying the collision energy. The fragment ions were monitored, and the collision cell energy and declustering potential were changed to select the fragment ion and collision cell energy which provided stable and intensive signal for detection and quantification. The MRM channels and optimized parameters for the detection and quantitation of all phenolic compounds are listed in Table 2-11.

Table 2-11. MRM channels and optimized parameters for the detection and quantitation of phenolic compounds.

	2,4-DNP	**Phenol**	**2,4,6-TCP**	**PCP**
Structure				
Retention time (min)	0.95	1.40	5.67	9.27
Molecular mass (Da)	182.9	93.0	196.8	264.8
DP (V)	60	60	60	80
MRM (Quantifier)	182.9 → 108.9	93.0 → 64.9	196.8 → 34.9	264.8 → 201.8
CE 1 (V)	-33	-30	-50	-40
CXP 1 (V)	-10	-20	-15	-15
MRM (Qualifier)	182.9 → 94.9	93.0 → 41.0	196.8 → 122.9	264.8 → 229.8
CE 1 (V)	-30	-50	-35	-35
CXP 1 (V)	-10	-15	-15	-15

A typical MRM chromatogram for the detection of four other phenolic compounds in standard solutions, along with chloroxylenol is shown in Figure 2-17. Each standard solution was spiked with 500 µg/L IS and the time required for each sample to complete the run was around 13 minutes. Sharp peaks with strong ion signals were observed for all compounds, while the peak area for IS, which was at fixed concentration of 500 µg/L, did not change significantly between different standard solutions.

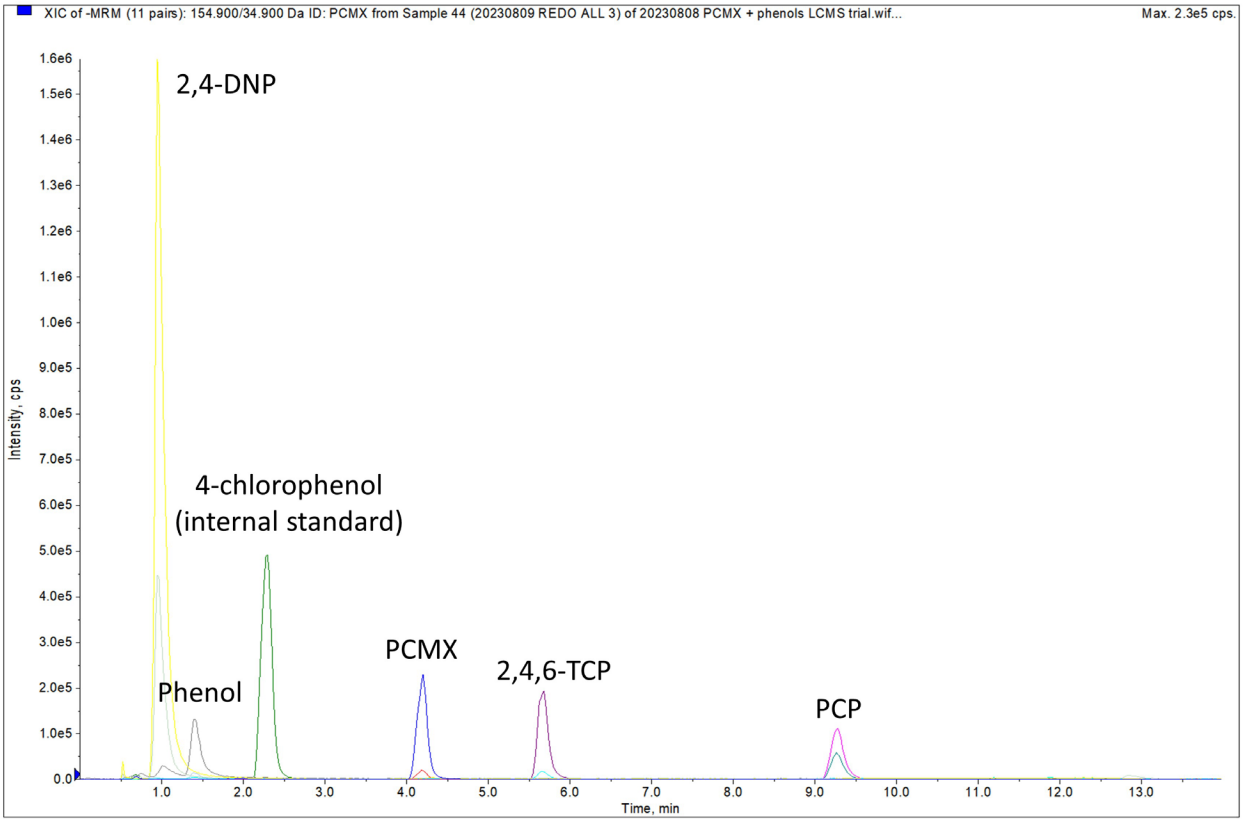


Figure 2-17. Typical MRM chromatogram of a standard solution with five phenolic compounds and 500 µg/L IS.

A typical MRM chromatogram for the detection of four phenolic compounds in river water samples collected in Yuen Long Creek is shown in Figure 2-18. Only chloroxylenol was detected and quantified. The peak area for IS, which was at fixed concentration of 500 µg/L, did not change significantly between different river water samples.

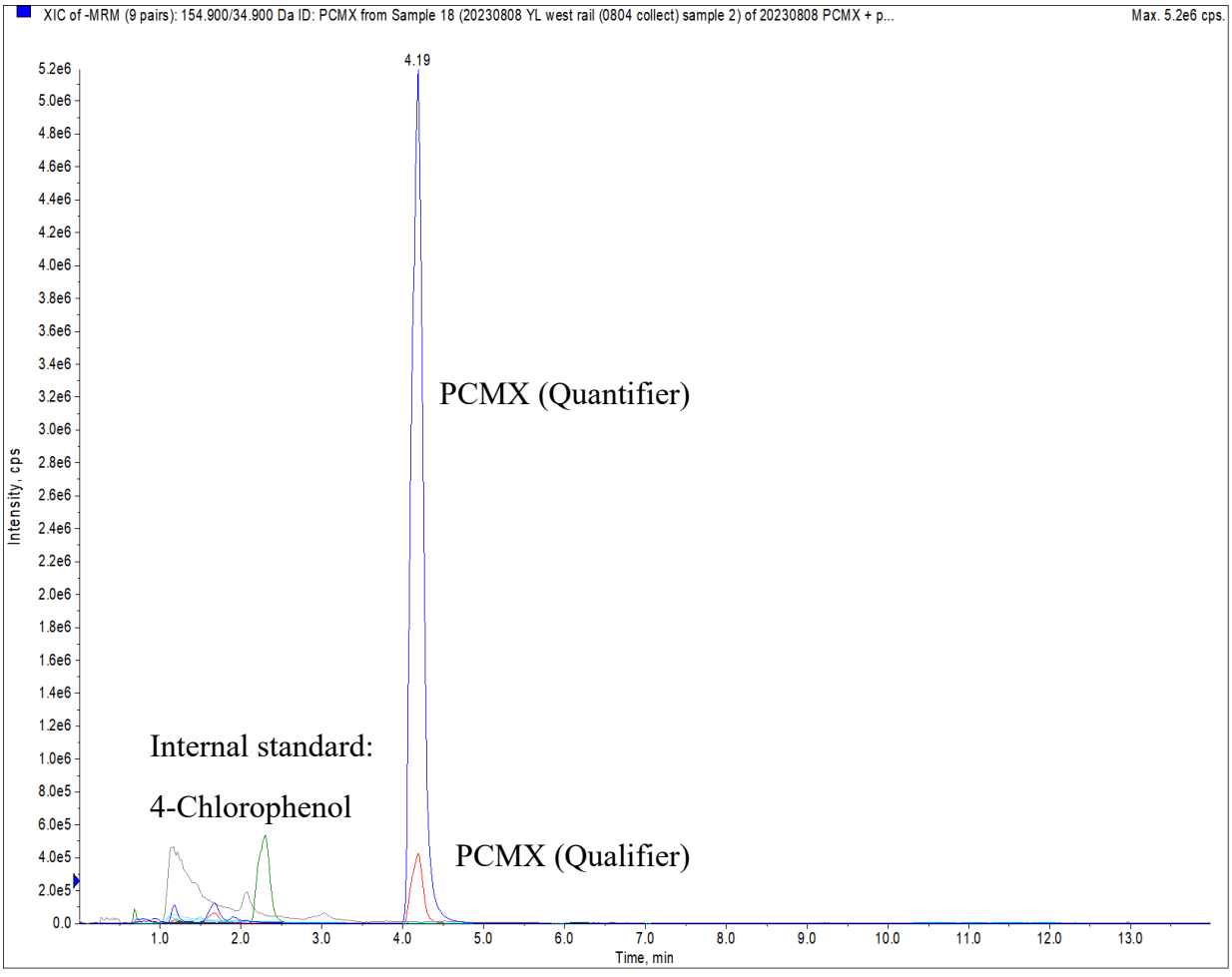
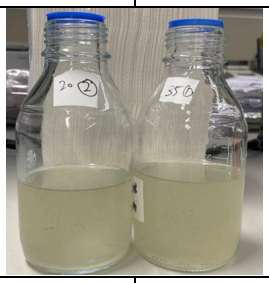


Figure 2-18. Typical MRM chromatogram obtained for a river water sample from Yuen Long Creek. Only chloroxylenol was detected and quantified.

2.3.5 Occurrence and concentrations of chloroxylenol and other phenolic compounds in wastewater of Hong Kong

Influent and effluent wastewater samples were collected at two STPs with different treatment processes during the study, using the same protocol in collecting and preserving river water samples. Two sampling campaigns were conducted for each STP respectively, and the results for each sampling campaign at the two STPs are shown in Table 2-12.

Table 2-12. Results from analyzing the influent and effluent wastewater samples collected at two STPs with different treatment processes.

		Sha Tin Sewage Treatment Works		**Stonecutters Island Sewage Treatment Works**	
		Campaign #1	Campaign #2	Campaign #1	Campaign #2
Sampling date		September 12, 2023		September 22, 2023	
pH of influent		6.45	6.48	6.54	6.52
pH of effluent		6.71	6.75	6.72	6.65
Photos of examples of influent samples					
Concentrations (µg/L)	influent	66.2 ± 5.3	104.0 ± 12.0	148.6 ± 21.5	120.4 ± 37.8
	effluent	Not detected	Not detected	107.1 ± 18.8	107.9 ± 20.7
Removal efficiency (%)		100	100	27.9	10.4
Concentrations (µg/L)	2,4 -DNP	Not detected		Not detected	
	Phenol				
	2,4,6-TCP				
	PCP				

Stonecutters Island Sewage Treatment Works and Sha Tin Sewage Treatment Works were selected in this study as they use different treatment processes, i.e., primary treatment (chemically enhanced sedimentation) and secondary treatment (aerobic biological treatment), respectively. They also serve nearly 90% of the Hong Kong total population. For Sha Tin Sewage Treatment Works, the removal efficiencies in the two sampling campaigns were both 100%, meaning that chloroxylenol is being completely removed by the microbial activities during the biological treatment. For Stonecutters Island Sewage Treatment Works, the removal efficiencies in two sampling campaigns were 27.9% and 10.4% respectively, achieving only a moderate level of effectiveness.[46] As chloroxylenol has a low partition coefficient of 3, removal through adsorption onto solid particles during primary treatment in sedimentation tanks is low, allowing a great portion of chloroxylenol to be discharged indirectly to the rivers and seas. The RQ values were over 20, which strongly proves that chloroxylenol is a potential stressor in Stonecutters Island Sewage Treatment Works and possess a high ecological risk to aquatic organisms. This is important as the removal efficiencies can allow us to determine the final output into the water environments such as surface water, groundwater, wastewater, seawater and drinking water. In summary, chloroxylenol can be removed through biodegradation,[47] but the removal of chloroxylenol is very limited in Stonecutters Island Sewage Treatment Works which relies on adsorption (i.e., sedimentation). As this STP treats the sewage of 80% of Hong Kong population, it should be upgraded to biological treatment in order to reduce the harm of chloroxylenol to the water environments of Hong Kong.

2.4 Conclusions

Chloroxylenol, a halogenated phenolic derivative, is one of the PPCPs that are reported to be moderately toxic to aquatic invertebrates and highly toxic to freshwater fish.[71] It is an active antimicrobial ingredient in various over-the-counter antibacterial hand sanitizers, cleaning solutions and household hygiene disinfectants from top-selling brands such as Dettol, Walch, Watsons and Ariel. Due to the extensive use of these products in homes and public settings during the COVID-19 pandemic, large amounts of chloroxylenol would be released continuously into the water environments (surface water, groundwater, wastewater, seawater and drinking water) through diverse routes.

As Hong Kong is highly populated with massive use of antibacterial hand sanitizers and household disinfectants, concentrations of chloroxylenol in rivers and wastewaters of Hong Kong during and after the COVID-19 pandemic might be much higher than those reported in earlier studies. However, chloroxylenol is not included in the routine river water quality monitoring programme implemented by the Government. In the beginning of the research study, diurnal studies were first conducted by collecting water samples at the sampling point in each river every hour to determine an optimized sampling time. It was noted that there was a significant difference in the concentrations of chloroxylenol between the two low tides; the concentrations of chloroxylenol at

the second low tide was much higher due to domestic human activities, indicating that for the remaining studies, if the extent of contamination was studied, the sampling should be conducted at a sampling timepoint when the second low tide was taken place instead of the first low tide.

In Yuen Long Creek, the highest concentration of chloroxylenol was 140 μg/L, which was measured on January 15, 2022 (Saturday). The highest concentration was correlated to the fifth wave of COVID-19 in Hong Kong. For the two sampling campaigns in Yuen Long Creek (January 12 to 22, 2022 and April 13 to 21, 2022), all RQ values obtained were higher than 1, while the maximum RQ value were over 25, which strongly proves that chloroxylenol is a potential stressor in Yuen Long Creek and possess a high ecological risk to aquatic organisms. Four typical phenolic compounds (phenol, 2,4-DNP, 2,4,6-TCP and PCP) were not detected in any of the river water samples collected in the two rivers. The results suggested that proper river management and regulation are necessary to improve river water quality and protect aquatic organisms in Yuen Long Creek.

Influent and effluent wastewater samples were also collected at two STPs with different treatment processes during the study, using the same protocol in collecting and preserving river water samples. For Sha Tin Sewage Treatment Works, the removal efficiencies of chloroxylenol in the

two sampling campaigns were both 100%, meaning that chloroxylenol is being completely removed by the microbial activities during the biological treatment. For Stonecutters Island Sewage Treatment Works, the removal efficiencies of chloroxylenol in two sampling campaigns were 27.9% and 10.4% respectively, achieving only a moderate level of effectiveness. In summary, chloroxylenol can be removed through biodegradation,[47] but the removal of chloroxylenol is very limited in Stonecutters Island Sewage Treatment Works which relies on adsorption (i.e., sedimentation). To prevent the introduction of chloroxylenol into the environment and preserve public health and safety, Stonecutters Island Sewage Treatment Works which treats the sewage of 80% of Hong Kong population should be upgraded to biological treatment in order to reduce the harm of chloroxylenol to the water environments of Hong Kong. The collected data is valuable for determining whether continuous sampling monitoring programs are necessary to address the environmental contamination of chloroxylenol in water environments.

Chapter 3: A comparative evaluation of the effectiveness of fire extinguisher dry powder to develop latent fingermarks

3.1 Introduction

Fingermarks, which consist of distinct ridges and valleys, have been used as a powerful personal identification tool for over a century, as the friction ridge pattern of each fingermark is unique to an individual and it remains unchanged throughout the lifetime.[86-88] Since being proposed in 1880, fingermarks left by an individual at a crime scene have been compared to reference fingerprints taken under controlled conditions.[89] Following the generation of pattern systems and databases for fingermarks to be classified and searched, fingermark comparison and identification have been successfully employed for over 120 years in criminal investigations, with an aim to confirm or reject the association of a suspect to a crime scene.

With the increasing awareness of criminal suspects, latent fingermarks, which are invisible to the naked eyes, are the most common types of fingermarks found at crime scenes. They are deposited when fingertips come into contact with various surfaces, thus allowing fingermark residues to form unique patterns during contact. To make the ridge details visible in these fingermarks, various physical and chemical fingerprint development methods are employed onto the fingermarks at crime scenes.

Powder dusting is one of the oldest and most widely used physical techniques for developing fingermarks on non-porous surfaces which allows finely divided powder to mechanically adhere to the moisture and oily components of friction ridge deposits, thus providing contrast between the fingermark features and the background substrate.[86] Although it has been used since the 19th century, the technique has limitations such as high toxicity, substantial background interference, poor contrast, limited selectivity and low sensitivity.[90] Development of new fingerprint powder as non-toxic, safe and simple alternatives remains of a major research interest in the field of forensic science, e.g., turmeric powder (a common ingredient in Indian food),[91] Robin powder blue (a common fabric-whitening agent),[92] durian seed powder[93] and marble slurry powder.[94] Similar to the conventional fingerprint powders, these new powders are destructive as all of them require brushes that may allow over 10 % of the latent fingermarks developed on site to be unidentifiable, due to damages to the fingermarks and the ridge characteristics caused by the brush directly contacting the fingermarks.[95] Especially when processing large crime scenes, powder dusting is very time-consuming, posing health problems to the crime scene personnel because inhalation of powders with particles smaller than 10 μm can lead to cardiovascular disease, cancer, asthma and other respiratory diseases.[96] The effect is even more pronounced with particles smaller than 2.5 μm, which corresponds to fine particulate air pollution.[96]

Cyanoacrylate fuming, commonly referred to as the superglue fuming method, is extensively utilized as a chemical technique to develop latent fingermarks on non-porous surfaces. Cyanoacrylate is warmed to form fumes that undergo polymerization when they interact with certain eccrine components (water, alcohol and amines) of latent fingermarks, thus yielding a white fingermark; thus, it has been reported to be more effective for eccrine fingermarks than for sebaceous fingermarks.[97-98] Large evidence items are typically packaged and transferred back to the laboratory, where latent fingermarks are developed using cyanoacrylate fuming in a cabinet with a proper ventilation system, but the development process is time-consuming and laborious, while the cyanoacrylate vapor produced is highly toxic which can cause irritation to the eyes and respiratory tract, and the fingermarks are prone to destruction during transportation. Cyanoacrylate fuming is generally followed by a post-treatment such as depositing a luminescent dye to further enhance the contrast and visibility of the fumed fingermarks. However, this additional step prolongs the processing time and is associated with significantly higher costs as complex and labor-intensive preparations, as well as an alternate light source are required to observe the developed fingermarks.

The size of a crime scene where a crime takes place differs from one scene to another and can vary significantly depending on the nature of the crime committed. For example, a crime scene

involving a homicide may encompasses multiple rooms within a house or building. As time passes during the processing of a crime scene, there is an increased risk of evidence disturbance or contamination by the crime scene personnel, victim, witnesses and bystanders. Crime scenes which are not managed in a timely manner would lead to either loss of evidence or poor-quality evidence resulting in erroneous exonerations or convictions in a criminal justice system.[99] Nowadays more than fifty fingermark development methods can be used to visualize latent fingermarks at crime scenes for documentation and comparison purposes, however over half of the fingermarks are not detected or identified, particularly when processing large crime scenes and handling bulky evidence items with large surface areas.[100-101]

The use of dry powder from a fire extinguisher (known as ABC powder) to detect latent fingermarks was first suggested in 2007, in which dry powder from a fire extinguisher outperformed water and foam fire extinguishers and revealed fingermarks of good quality.[102-103] The theory of using fire extinguisher dry powder was then verified by developing over 50% of fresh fingermark impressions with excellent quality, contrast and exceptional detail on various non-porous surfaces commonly found within marijuana growing operations.[104] Specifically, 81.25% of the deposited fingermarks were successfully developed on a glass pane (window), whereas only one out of the sixteen impressions were visible on metal foil insulating tape.[104] In

2016, the development of fingermarks with fire extinguisher dry powder was found to be comparable to conventional methods on both heated and unheated glass and tile surfaces, but not on metal surfaces; therefore, the authors proposed a mechanism by which the fire extinguisher chemicals bind to the metal ions present in the fingermarks.[105] Although first suggested in 2007, no exhaustive application and comparison experiments have been presented to evaluate the performance of fire extinguisher dry powder in fingermark detection. The characterization of ABC powder and the exact mechanism by which the chemical reacts with the fingermarks has not been studied sufficiently. Fresh and sebaceous fingermarks were used in all the mentioned studies, which did not mimic typical casework scenarios; therefore, aged and natural fingermarks (i.e., fingermarks with secretions naturally found on the donor's fingers) should also be evaluated. Despite being commercially available, the powders have been the focus of only a very small number of studies, limited to only one donor and a few deposited fingermark samples or substrates, and thus further research is needed to investigate the performance and advantages over common techniques. Moreover, split-fingermark results or statistical analysis were not included in the previous studies.[102-105]

In terms of crime scene use, the relatively low cost of an ABC-type dry chemical fire extinguisher makes it significantly more amenable as a practical tool for crime scene investigators. Spraying the entire crime scene can be done in a few seconds without any search examination. The risk of destroying the fingermarks is minimal as evidence items do not need to be transported to a laboratory for cyanoacrylate fuming. The technique of using fire extinguisher dry powder as a novel "*in situ*" development powder could be proved to be cheap, simple, safe, efficient and less labour-intensive. The aim of this study was to characterize different brands of ABC powder by particle sizes, morphology and elemental composition, and to compare the selectivity (ability to develop homogenous fingermark ridges with fine details), sensitivity (ability to develop visible fingermarks even with weak deposits) and stability (ability to develop visible fingermarks which have been aged for a period of time) in detecting latent fingermarks with those of the current fingermark development methods using traditional fingerprint powders and cyanoacrylate fuming in an in-depth and systematic way. The results would help forensic scientists, criminologists and other investigators to make an informed choice when selecting a detection technique for 'in situ' latent fingermarks when working on criminal cases.

Objectives of the research study:

a) To characterise fire extinguisher dry powder by particle sizes, morphology and elemental composition;

b) To provide insight into the mechanism of fire extinguisher dry powder in detecting various types of latent fingerprints (with eccrine, sebaceous and natural residues) in terms of selectivity and compare with two common routine fingerprint development methods;

c) To determine the sensitivity of fire extinguisher dry powder by developing depleted latent fingerprints and compare with two common routine fingerprint development methods;

d) To determine the stability of fire extinguisher dry powder by developing latent fingerprints on glass surfaces stored for periods up to 24 weeks and compare with two common routine fingerprint development methods.

3.2 Experimental section

3.2.1 Study design

This study was completed in four parts to conduct a full evaluation of the use of fire extinguisher dry powder to detect latent fingermarks (Figure 3-1). The first part focused on the characterization of different brands of ABC powder and traditional fingerprint powders to determine the particles sizes, morphology and elemental composition using scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX), as the size and shape of the powder particles affect the quality and number of developed fingermarks. This study focused only on fire extinguishers that are approved by the Hong Kong Fire Services Department for use in commercial and residential settings. The second part was a comparative study to investigate the possible mechanism of powder to detect latent fingermarks with different residue compositions on four types of glass substrates. The effectiveness in detecting latent fingermarks was compared with current fingermark development methods. Details of the variables used within this mechanism study can be seen in Table 3-1. At last, the sensitivity of the powder to various fingermarks containing decreasing amounts of residue deposited was investigated using spilt fingermarks for direct comparison of the development methods from the same fingermark deposition. Fingermarks were also stored for different time intervals to evaluate the stability of each method. Overall, experimental parameters (e.g., number of fingermark donors, depletion series, substrates,

development methods, types and age of fingermarks) were assessed to obtain a realistic assessment of each method's performance.

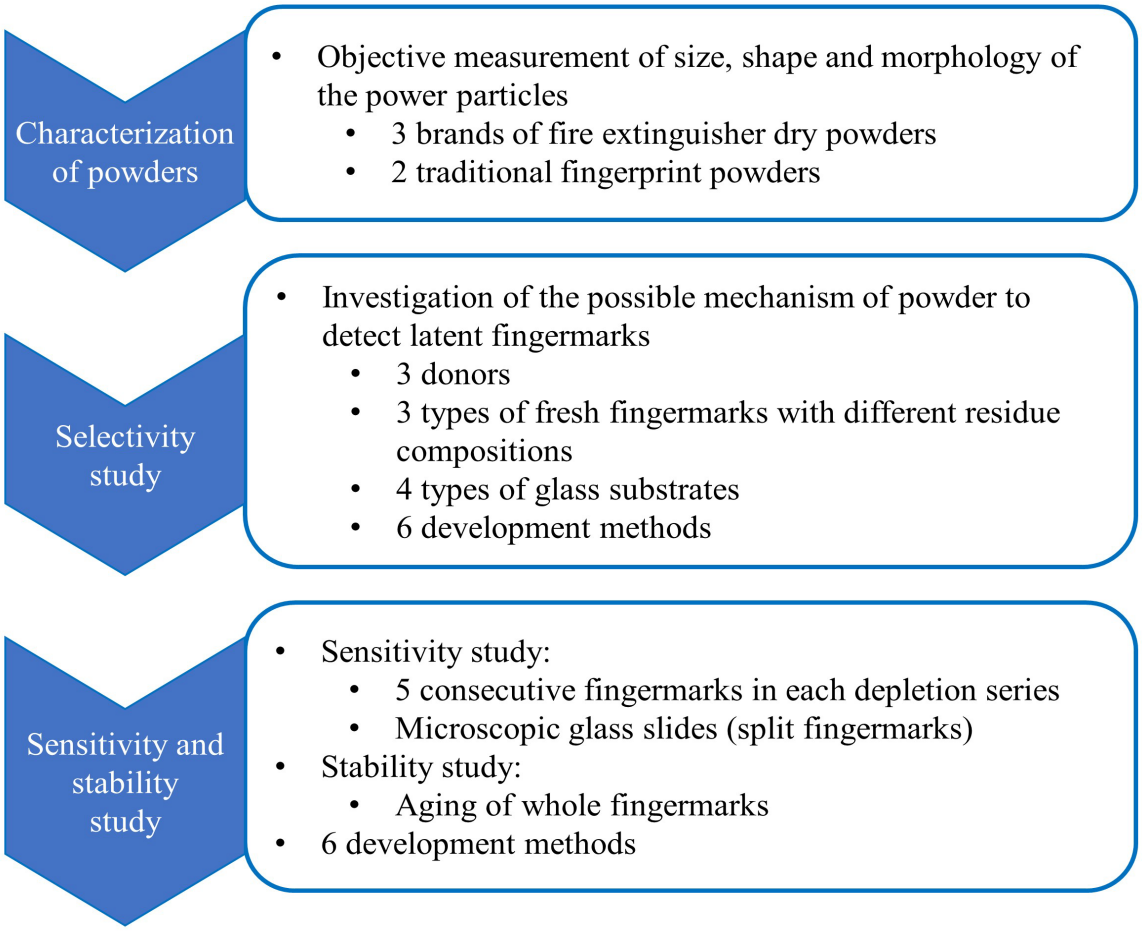


Figure 3-1. Experimental design of the study.

Table 3-1. Summary of the parameters tested within the selectivity study.

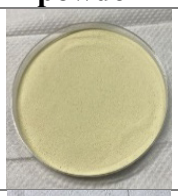
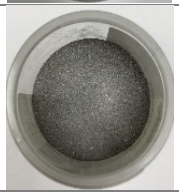
		Total number of fingermarks
3 donors		3
3 types of fingermarks	Natural	9
	Sebaceous	
	Eccrine	
Repeat for 3 times		27
4 types of glass substrates	Clear float glass	108
	Tinted glass	
	Tempered glass	
	Mirror glass	
6 development methods	Three different brands of fire extinguisher dry powder	648
	Two traditional fingerprint powders	
	Cyanoacrylate fuming	

3.2.2 Chemicals and materials

Fire extinguisher dry powders and traditional fingerprint powders

Table 3-2 presents a selection of three commercially available fire extinguishers and two traditional fingerprint powders used in this study. This study focused only on fire extinguishers that are approved by the Hong Kong Fire Services Department for use in commercial and residential settings.

Table 3-2. Fire extinguisher dry powders and traditional fingerprint powders tested.

Types of powder	Supplier	Country of origin	Color of powder
Fire extinguisher ABC dry powder	Eversafe	Malaysia	
	SRI	Malaysia	
	VSJ	China	
White latent fingerprint powder	SceneSafe	United Kingdom	
White magnetic fingerprint powder	SceneSafe	United Kingdom	

Substrates

Considering the prevalence of glass encountered at different crime scenes involving burglary, housebreak and theft in which latent fingermarks should never be overlooked, four types of glass substrates were obtained from retail and commercial suppliers in order to assess if certain development methods were more suitable for particular substrates. Table 3-3 lists the information of each glass substrate. All the surfaces were cleaned prior to deposition by wiping the surface with methanol and were only be handled while wearing gloves.

Table 3-3. Selection of glass substrates for the selectivity study.

Types	Description	Applications
Clear float glass	• Transparent • Extreme clarity • Makes up about 90% of all manufactured glass	Windows of buildings, car windows, doors
Tinted glass	• Darker appearance • Solar heat reduction	Windows in buildings and cars
Tempered glass	• Transparent • Extreme clarity • Much stronger than regular glass	Car windows, shower doors, glass tables
Mirror glass	• Highly reflective	Vehicle mirrors, furniture tops, exterior facades

3.2.3 Characterization of powders

The particle sizes and morphology of the powder particles present in three commercially available fire extinguishers and two traditional fingerprint powders were determined using a Jeol JSM-6490 scanning electron microscope (SEM). To analyze each sample, powders were deposited onto a silicon chip wafer that had been glued to an aluminum SEM stub with conductive double-sided adhesive carbon tape. The stubs were then sputter-coated with gold and mounted onto the sample stage of the SEM, allowing the sample to be viewed and imaged with Oxford INCA-X Manuals Software. These images were then analyzed using the ImageJ software to determine the average particle size and size distribution profile of each type of powder by calibrating the scale using the imbedded scale in the SEM images. Elemental composition analysis for each powder was also

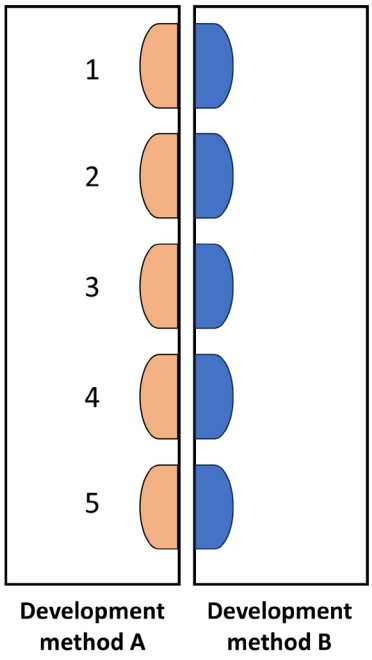
studied by an energy dispersive X-ray spectroscopy (EDX) detector, with each element and the corresponding atomic composition being quantitatively measured.

3.2.4 Latent fingermark deposition

In the selectivity study, to explore the reaction mechanisms of fire extinguisher dry powder, three types of fingermarks (natural, eccrine and sebaceous fingermarks) were deposited on four types of glass substrates by three healthy individuals, two males and one female with their age ranging from 20 to 30 years old, who were selected to represent strong, average and weak fingermark donors. For natural fingermark deposition, donors were asked to wash their hands and then pursue their normal routine activities for one hour so as to mimic a real-life scenario.[106] They were asked to rub their hands together to get an even coating of natural secretions and/or contaminants across the fingers before depositing fingermarks, allowing adequate residues to collect naturally on the fingers. The fingers were placed onto each substrate with moderate force for three seconds. For eccrine fingermark deposition, after thoroughly washing, rinsing and drying the hands, the hands were sealed in clean polyethylene bags for thirty minutes to allow them to sweat, after which the donors were asked to place the fingers onto each substrate with moderate force for three seconds. For sebaceous fingermark deposition, donors were asked to thoroughly wash, rinse and dry the hands to remove any greasy matter. After rubbing the fingers on oily regions such as the nose, cheeks or forehead, the fingers were placed onto each substrate with moderate force for three

seconds. The sebaceous content in the residues would increase substantially, thus deviating from the chemical composition of other fingermarks.

In the sensitivity study, five fresh successive depositions of the same finger were made by a female donor without replenishment to obtain a depletion series of fingermarks. With each deposition, progressively fewer quantities of material were deposited, forming a sequential study. The fingermarks were deposited across two adjacent microscopic glass slides as split marks where each half of a fingermark was developed using a different development method. This allowed for direct comparison of the development methods from the same fingermark deposition. This was repeated between each detection method forming six sets as shown in Figure 3-2. In the stability study (i.e., time study), natural and sebaceous fingermarks were deposited on microscopic glass slides by three healthy individuals, who represented weak, average and strong fingerprint donors. All the fingermarks were stored in laboratory drawers with temperature and relative humidity ranges of 18-22 °C and 40-70% respectively for nine ageing periods of 6 hours, 24 hours, 72 hours, 1, 2, 4, 8, 12 and 24 weeks before visualizing with one of the six development methods. All experiments were performed in triplicate.



Development method A	Development method B
Eversafe ABC powder	White magnetic powder
Eversafe ABC powder	White latent powder
SRI ABC powder	White magnetic powder
SRI ABC powder	White latent powder
VSJ ABC powder	White magnetic powder
VSJ ABC powder	White latent powder

Figure 3-2. Diagram of split fingermark deposition with direct comparisons between all development methods, where method A and B were used on either side of each fingermark.

3.2.5 Latent fingermark development

To investigate the selectivity, sensitivity and stability in detecting latent fingermarks, three different brands of fire extinguishers were compared with two traditional fingerprint powders and cyanoacrylate fuming.

Fire extinguisher ABC dry powders

As with traditional application, fire extinguisher dry powders were slowly sprayed from each cylinder with the following procedures:

i. Remove safety pin.

ii. Hold unit upright and aim nozzle at the substrate from three feet.

iii. Squeeze trigger to dispense the pressurised white dry chemical powder.

iv. Allow ten minutes for the powder to settle before examining fingermark development.

v. Use a pipette bulb filler to puff away any excess powder on the substrate if needed.

Traditional fingerprint powders

White magnetic fingerprint powder was applied by means of a magnetic fingerprint brush (SceneSafe, UK), while white magnetic fingerprint powder was applied with a zephyr fibre fingerprint brush (SceneSafe, UK). The brush was loaded with powders with care and then applied to the substrates, following the direction of ridge flow until the fingermark was fully developed.

Cyanoacrylate fuming

The heat and humidity method was employed using a cyanoacrylate fuming cabinet, with the following parameters chosen for fingermark development: 0.5 g of superglue, with the chamber set to fume for 50 min at 100 °C and at 80% relative humidity. All substrates were placed vertically from the top rail for a consistent fingermark development. The door was closed during the fuming process, allowing the substrates to be exposed to the fumes. After the procedure was complete, the substrates were removed from the cabinet to view any visualized fingermarks.

3.2.6 Grading of developed fingermarks

Each developed fingermark was photographed under visible light as soon as possible after development and graded without prior information as to the development method to avoid bias in the data. A grade from 0 to 4 was assigned for quality and clarity using the UK Home Office Centre for Applied Science and Technology (CAST) scale to determine their suitability for comparison and identification purposes shown in Table 3-4.[107]

Table 3-4. CAST scale used for fingermark assessment.[107]

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

3.2.7 Fingermark analysis

Statistical analysis of the mean values of the fingermark grading data was carried out using IBM SPSS® Version 29 software to assess if there was a significant difference in the grading of developed marks for different development methods, glass substrates, ageing periods and types of fingermarks using repeated measures ANOVA tests. Bonferroni multiple comparison testing was employed and a value of $p \leq 0.05$ indicates a significant difference in the fingermark gradings within the data sets at a 95% confidence level.

3.3 Results and discussion

3.3.1 SEM/EDX characterization of powders

The chemical, physical and morphological composition of fingerprint powders is crucial in determining the adhesion capabilities on how the particles stick to the ridge patterns of latent fingermarks and consequently, the quality of the developed prints. Although various fingerprint powder can be purchased online or from manufacturers, these commercial powders are mainly distinguished by their visual characteristics without any labelled chemical identification, leaving forensic scientists unaware of the specific properties of the powders they are using. For fire extinguishers, narrow particle size distribution allows better flow properties, however, the minimal and maximal particle sizes are different for various brands and manufacturers, following the standards adopted by different countries.[108]

Figure 3-3 shows the surface morphology of the five powders taken at 500x magnification, along with the illustrations of histograms showing their corresponding particle size distribution. Particles from the three commercially available fire extinguishers were very similar in size, distribution and morphology. They consisted of particles less than 60 μm that were rough and irregular in surface morphology, with some particles exhibiting an oblong shape with very shape edges. Table 3-5 lists the estimated average particle sizes of each type of powder. For VSJ fire extinguisher dry powder,

the average particle size was found to be 14.7 µm, with a range of particle sizes varying from 5.7 to 50.5 µm. Similarly, Eversafe dry powder exhibited a comparable average particle size of 15.7 µm, but with a slightly wider range of particle sizes spanning from 5.6 to 59.2 µm. SRI dry powder contained particles with sizes between 5.7 and 57.3 µm with a consistent distribution of particles with a size between 7 and 28 µm. As a result, SRI dry powder had the largest average particle size among the three powders, measuring 20.0 µm. White latent fingerprint powder with a particle size range of between 7.2 and 33.8 µm had clean and smooth surfaces, where it could be seen that the discrete spherical particles had good dispersion and uniform particle size. A relatively narrow size distribution of the particles was also indicated. White magnetic fingerprint powder was composed of particles with irregular shapes characterized by complex surface contours and jagged edges. It had the largest average particle size among the five powders, measuring 36.9 µm. In summary, the three different brands of fire extinguisher displayed powder particles with very diverse shapes, from curved, irregular to sharp-edged, suggesting that the large surface area may facilitate better contact with fingermark deposits.

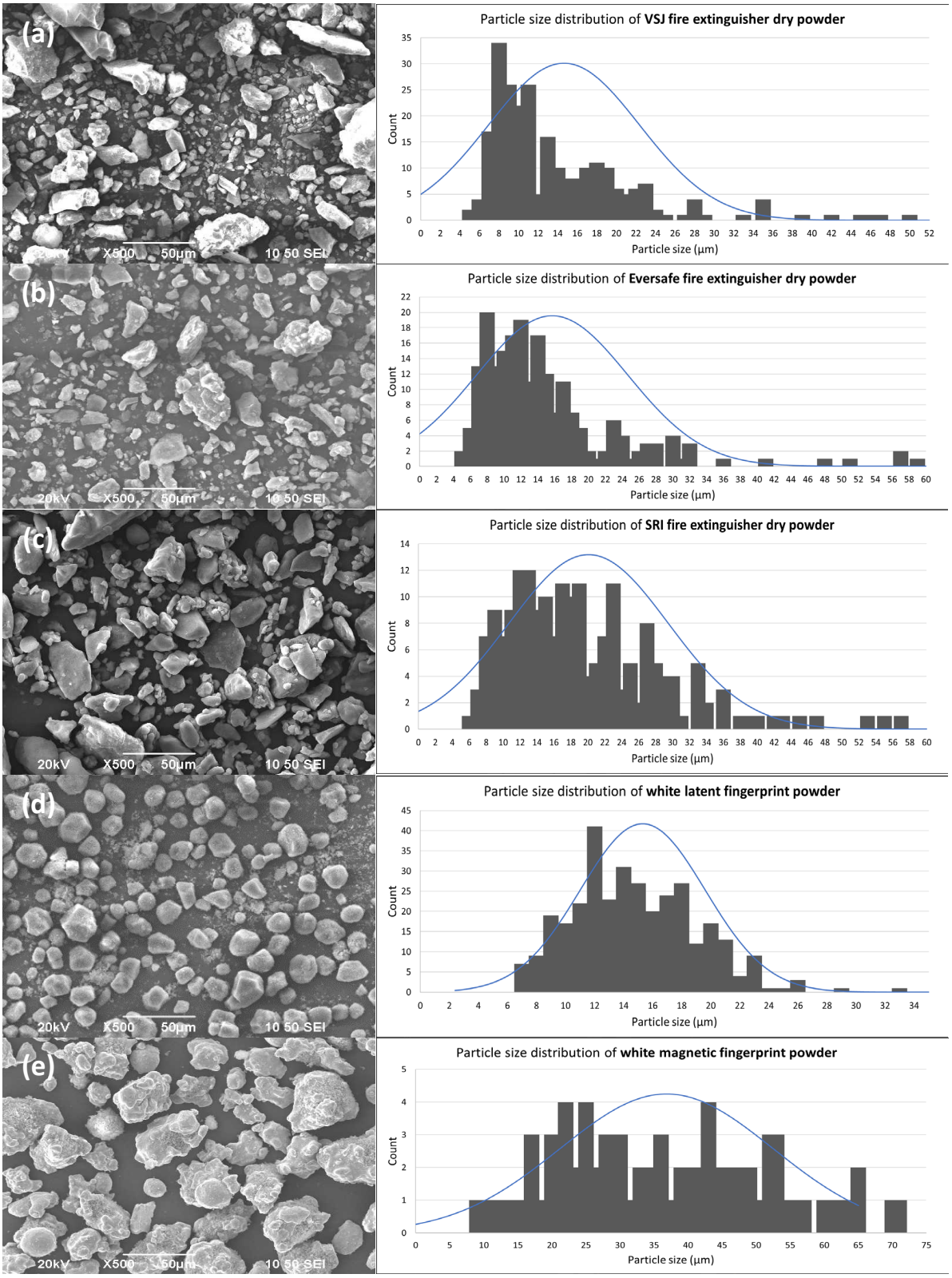


Figure 3-3. SEM image (left) and the particle size distribution analysis (right) of (a) VSJ fire extinguisher dry powder, (b) Eversafe fire extinguisher dry powder, (c) SRI fire extinguisher dry powder, (d) white latent fingerprint powder and (e) white magnetic fingerprint powder.

Table 3-5. Particle sizes of the five powders estimated using SEM.

Types of powder	Particle size (μm)
VSJ fire extinguisher dry powder	14.7 ± 7.7
Eversafe fire extinguisher dry powder	15.7 ± 9.0
SRI fire extinguisher dry powder	20.0 ± 9.4
White latent fingerprint powder	15.3 ± 4.3
White magnetic fingerprint powder	36.9 ± 15.7

The effectiveness with which the fingerprint powder adheres to the friction ridges for clarity depends on the size and shape of the powder particles. Small, rounded and well-dispersed particles in the range of 1 to 10 μm were found to adhere more easily than large, coarse ones.[109] A previous study indicated that fingerprint powders with particle sizes around 50 nm and uniform spherical morphology were the best powders for latent fingermark development, while powders with large particle sizes, irregular shapes and complex elemental compositions ranked poorly.[110] However, small-sized particles with sizes of ≤ 10 μm could enter through the respiratory system and accumulate in the lungs, while sizes of ≤ 2.5 μm could cause serious health problems by penetrating the skin or cell membranes and inducing cell death.[111] White latent fingerprint powders, which are titanium dioxide based, have also been linked to harmful threats and carcinogenic hazards towards the users over time.[112] The process of applying these fingerprint powders using a brush during forensic investigations can extend the time required for the task and consequently increase the potential health risks faced by forensic investigators. Based on this study, the three different brands of fire extinguisher dry powder examined were found to be large enough to be

effectively captured using face masks. Additionally, their particle sizes were deemed too large to pass through biological membranes such as skin and lung tissue. As a result, when following good practices, such as wearing personal protective equipment, these powders are considered safe for developing latent fingermarks. Most importantly, spraying the entire crime scene can be done in a few seconds without any search examination, which eliminates the laborious task of powdering the entire scene using a brush and reduces the potential health risks faced by forensic investigators.

ABC dry fire extinguishers typically contain monoammonium phosphate as the primary ingredient, either on its own or in combination with ammonium sulphate, depending on the manufacturers, brands and specific product models. Certain additives, such as organosilicon compounds, fumed and precipitated silica, metal stearates, nepheline, tricalcium phosphate, mica, talc, graphite, pyrophyllite, clay, talc and aluminum silicate may be added to enhance the flow, increase bulk density, minimize caking and improve the fire-fighting capacity.[113-116] Among the three fire extinguishers investigated in this study, the material safety data sheet is only accessible for Eversafe fire extinguisher, which indicates the presence of monoammonium phosphate (42%), ammonium sulphate (46%), mica (< 5%) and poly(methylhydrosiloxane) (PMHS) (< 1%).[117] Figure 3-4 shows the EDX spectra of white latent fingerprint powder and Eversafe fire extinguisher dry powder, which clearly indicated that the primary composition of the fire

extinguishing dry powder was monoammonium phosphate with many minor elements such as aluminum, silicon, magnesium, sodium and calcium.

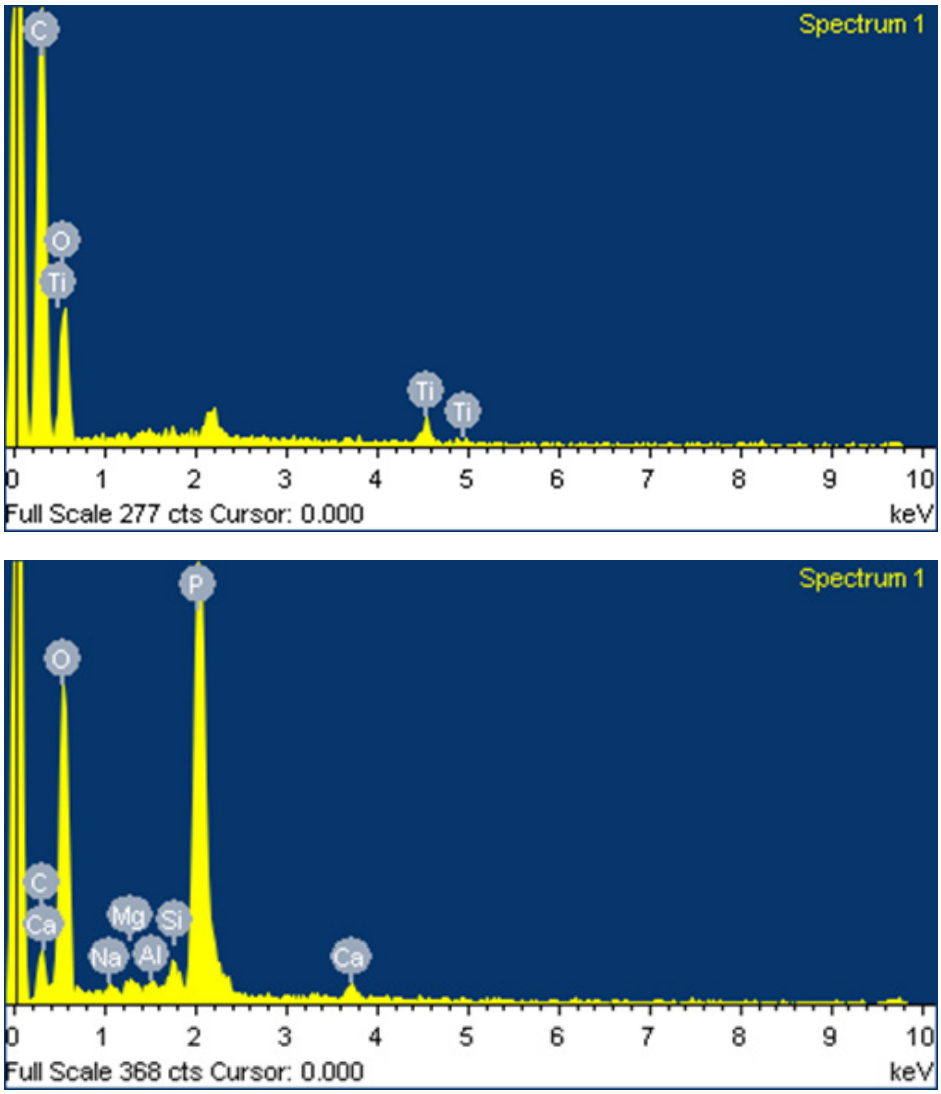


Figure 3-4. EDX spectra of white latent fingerprint powder (top) and Eversafe fire extinguisher dry powder (bottom). The unlabeled peak at 2.2 keV correspond to the Au layer added in the gold sputtering of the sample.

A comprehensive list of the elements detected in each powder is summarized in Table 3-6. In addition to the presence of mica and PMHS as noted on the material safety data sheet, there were

indications that calcium carbonate, sodium bicarbonate, magnesium hydroxide, sodium aluminosilicates and silica may also be present. These additives were also reported to be found in dry fire extinguishers.[118] From the quantification data, the three fire extinguisher dry powders could be grouped together with regards to their elemental composition. They were of identical composition, with similarities in the quantification data, where Eversafe fire extinguisher dry powder was found to be comprised of many minor elements such as aluminum, sodium and calcium. There were some variations in the concentrations of the elements present which could be attributed to the differences in manufacturers. The EDX analysis confirmed that the primary composition of the three fire extinguishing dry powders was monoammonium phosphate. In the Eversafe fire extinguisher dry powder, besides the presence of mica and PMHS as indicated on the material safety data sheet, it is suggested that additional components such as calcium carbonate, sodium bicarbonate, magnesium hydroxide, sodium aluminosilicates and silica may also be present.

Table 3-6. EDX elemental composition of the five powders.

Presence of element	**Fire extinguisher dry powder**			**Traditional fingerprint powder**	
	VSJ	**Eversafe**	**SRI**	**White latent**	**White magnetic**
Oxygen	✓	✓	✓	✓	✓
Titanium				✓	✓
Silicon	✓	✓	✓		✓
Aluminum		✓			
Iron					✓
Phosphorus	✓	✓	✓		
Magnesium	✓	✓	✓		
Sodium		✓			
Calcium		✓			

3.3.2 Selectivity of fire extinguisher dry powder for latent fingermarks (mechanism study)

When all donors (two males and one female), types of fingermarks (natural, eccrine and sebaceous fingermarks) and glass substrates were considered, Eversafe fire extinguisher dry powder yielded fingermarks of comparable quality to both white latent fingerprint powder and white magnetic fingerprint powder. Looking at the data overall, the mean grade of the Eversafe powder was 3.56, while the mean grades for white latent fingerprint powder and white magnetic fingerprint powder were 3.50 and 3.69 respectively. Representative images of different types of fingermarks developed with white latent fingerprint powder and Eversafe fire extinguisher dry powder and photographed under visible light are shown in Figure 3-5. All sebaceous fingermarks displayed a higher contrast regardless of the development method used due to the higher concentrations of moisture and oily components in the sebaceous secretions. The three brands of fire extinguisher dry powder showed higher selectivity to the fingermark residues; thus no reaction was observed with the glass surface. All sebaceous fingermarks developed with fire extinguisher dry powders were graded 4 with sufficient ridge detail and were categorized as “useful” for potential identification (grade of 3 or 4). Over 63% of deposited natural fingermarks (i.e., with natural secretions from glands in the skin and environmental contaminants), which are representative of the actual operational casework, were graded 3 or 4. Eccrine fingermarks that consist primarily of water were comparatively faint, but over 44% were still graded useful with well-defined ridge details and sufficient contrast with the background. Discernible friction ridges were clearly

observed when the fingermarks were developed with fire extinguisher dry powders, thus demonstrating the potential application of these powders in detecting latent fingermarks.

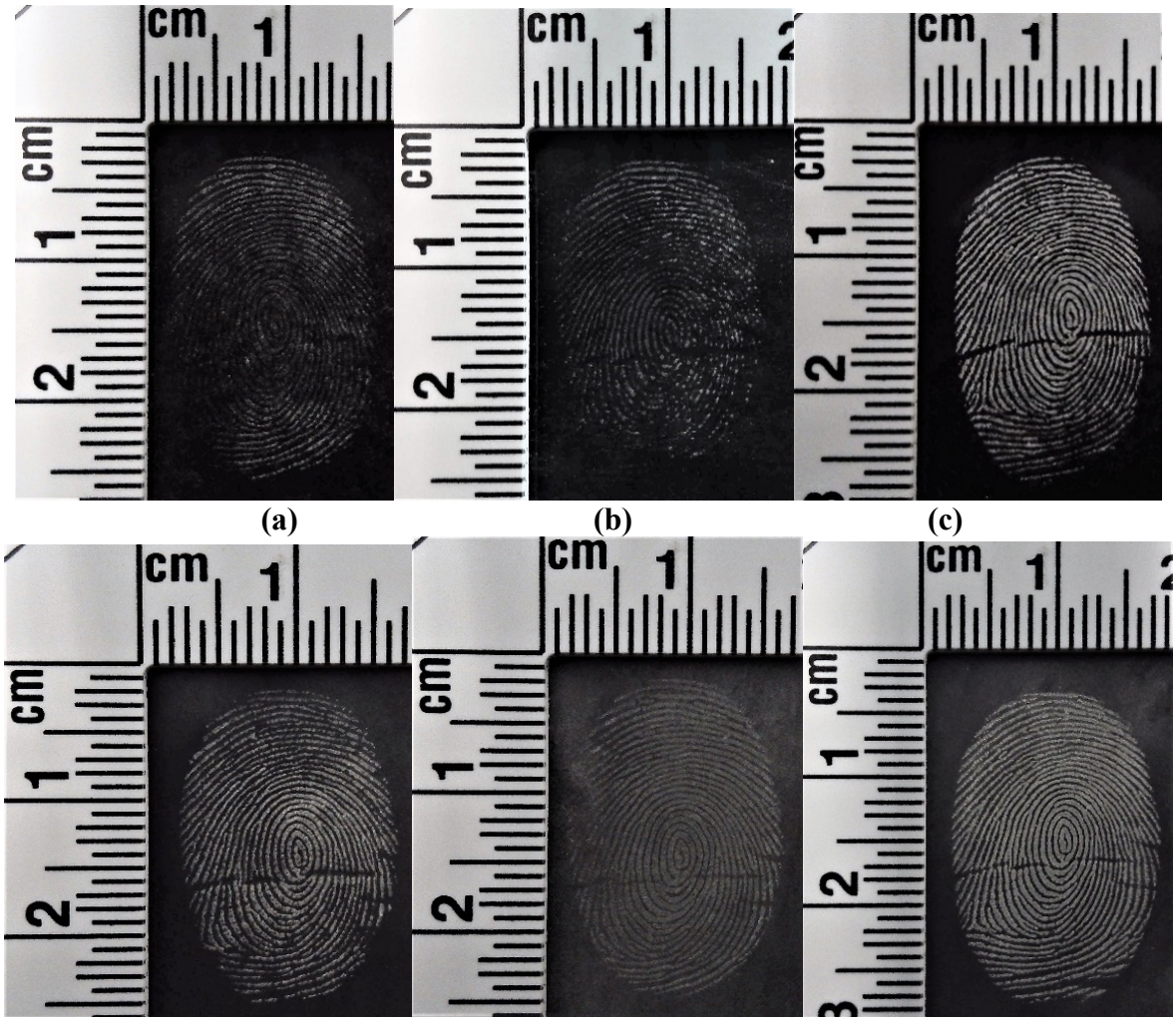


Figure 3-5. Representative images of a (a) natural, (b) eccrine and (c) sebaceous latent fingermark developed with white latent fingerprint powder (top) and Eversafe fire extinguisher dry powder (bottom) visualized under visible light on clear float glass.

For each fingermark development method, the mean grade was summarized across all donors and glass substrates for each type of fingermark in Fig 3-6. The results of the repeated measures ANOVA tests across the six development methods suggested that significant differences were present within the data for all six development methods except white latent fingerprint powder ($p \leq 0.05$), while the eta-squared results suggested that the magnitudes of the difference were large. Very small p values (in the range of 0 to 0.012) were obtained for the five development methods, indicating a significant difference in the mark gradings for each method at 95% confidence level. Bonferroni multiple comparison testing was then employed for assessing significant differences between pairings and a value of $p \leq 0.05$ indicates a statistically difference in the fingermark gradings within the data sets. Similar to the traditional fingerprint powders, Eversafe fire extinguisher dry powders displayed notable superiority in developing sebaceous and natural fingermarks by giving sufficient ridge details for potential identification (grade of 3 or 4).

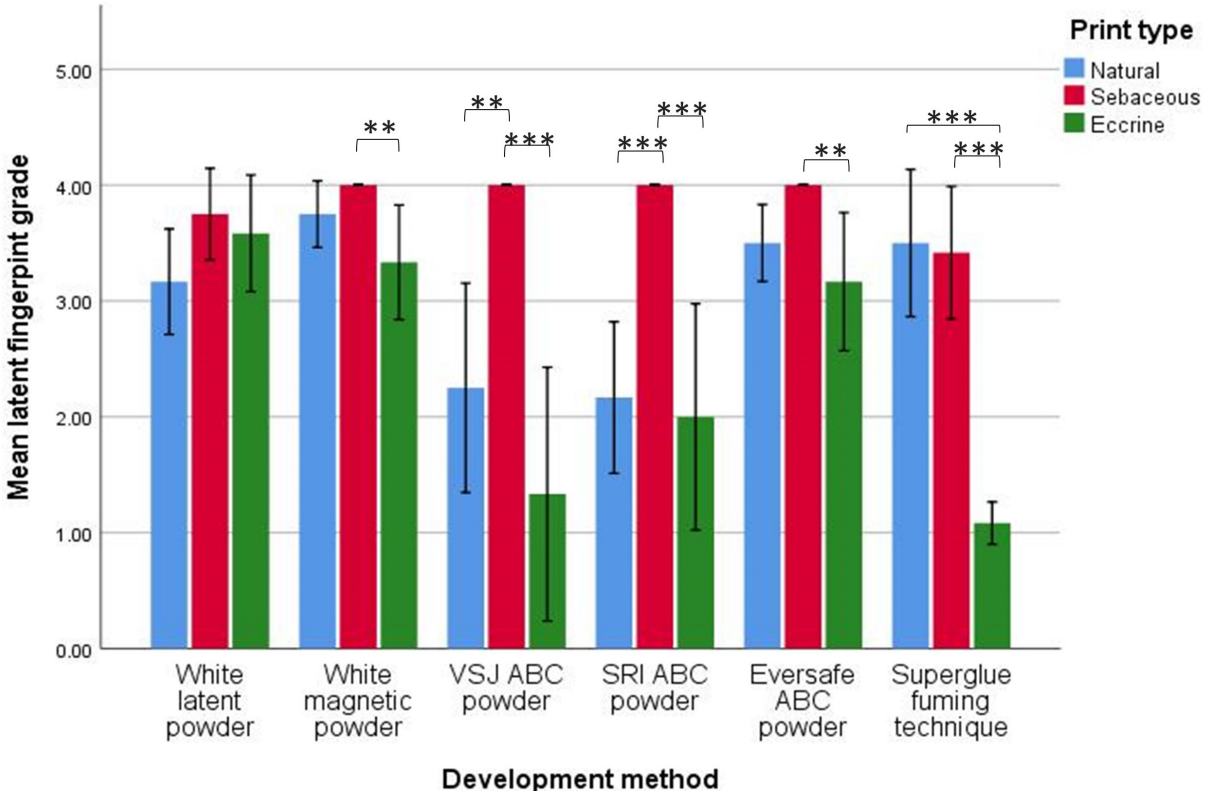


Figure 3-6. Comparison of the mean latent fingermark grades developed using six development methods for each type of fingermark.

Fingermark detection quality using fire extinguisher dry powders was evaluated on four types of glass substrates with different properties and applications. Fingermarks with excellent ridge details were obtained on all the glass substates by spraying with Eversafe fire extinguisher dry powder, with representative images of developed natural fingermarks being shown in Figure 3-7. To enhance the visibility of developed fingermarks on mirror glass using proper lighting techniques and angles, the reflective nature of the substrate may still present challenges to image the developed fingermarks. Despite these efforts, a mottled appearance of the developed fingermarks on mirror glass was still observed. The deposited natural fingermarks, which are representative of

actual operational casework, showed good ridge detail with minimal background staining on all glass substrates, thus demonstrating the good selectivity of fire extinguisher dry powders towards the fingermark residues.

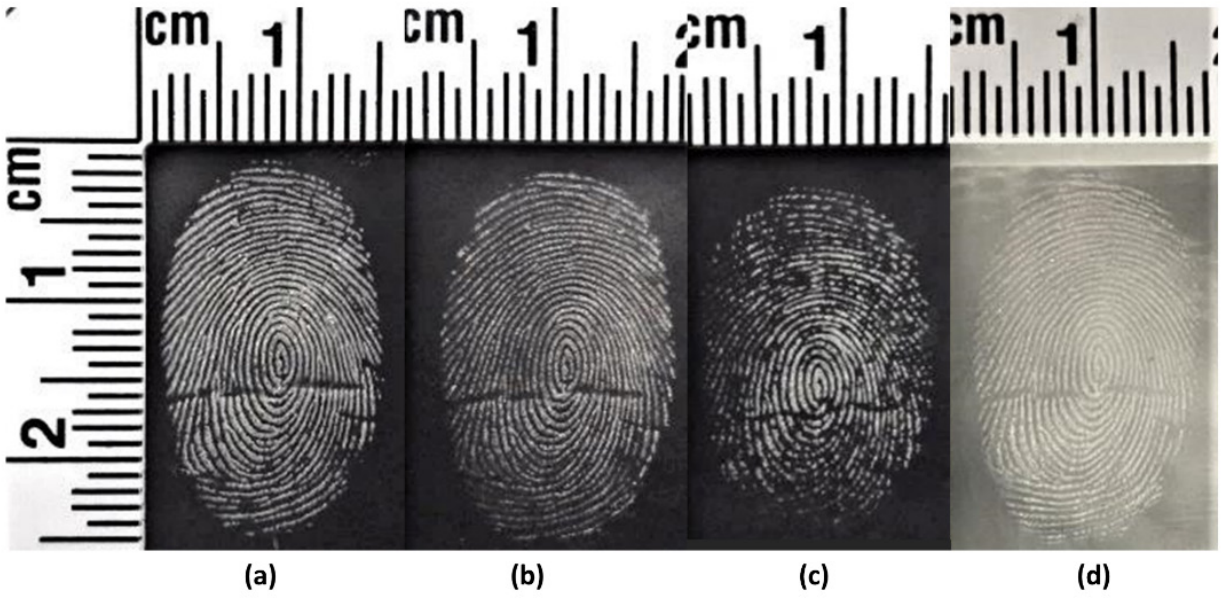


Figure 3-7. Representative images of natural latent fingermarks developed with Eversafe fire extinguisher dry powder (bottom) visualized under visible light on (a) clear float glass; (b) tinted glass; (c) tempered glass and (d) mirror glass.

On clear float glass, the latent fingermarks developed using Eversafe fire extinguisher dry powder exhibited the highest mean grade (mean grade of 4) among the development methods. This is noteworthy since clear float glass constitutes approximately 90% of all manufactured glass. However, it is important to mention that the other two brands of fire extinguisher, namely SRI (mean grade of 2.9) and VSJ (mean grade of 2.9), did not perform as effectively in developing fingermarks on clear float glass. Furthermore, white latent fingerprint powder and white magnetic

fingerprint powder outperformed the three brands of fire extinguisher in terms of fingermark development on tinted glass, tempered glass and mirror glass. However, over 85% of the fingermarks developed with Eversafe fire extinguisher dry powder on these three substrates were still graded 3 or 4. These findings are presented in Figure 3-8 and was taking into account all donors and fingermark types. These results demonstrated that quality of fingermark development could also be influenced by substrate.

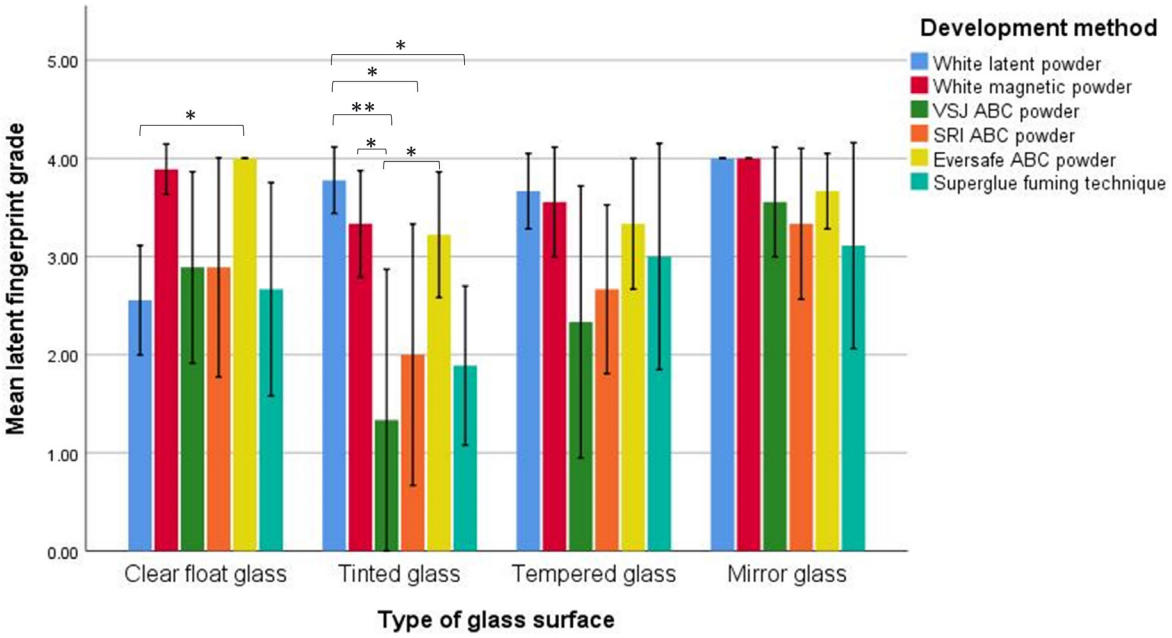


Figure 3-8. Comparison of the mean latent fingermark grades developed using six development methods for each type of glass substrate.

Overall, sebaceous fingermarks produced more defined ridge details than eccrine and natural fingermarks when developing with fire extinguisher dry powders. Similar to other traditional

fingerprint powders, spraying the latent fingermarks with fire extinguisher dry powders is a physical method of development that relies on the mechanical adherence of powder particles to the fingermark residues. As discussed earlier, the EDX analysis confirmed that the primary composition of the three brands of fire extinguisher was monoammonium phosphate, while additives such as mica, PMHS, calcium carbonate, sodium bicarbonate, magnesium hydroxide, sodium aluminosilicates and silica may also be present in the fire extinguisher dry powders. Taking PMHS as an example, this additive has two kinds of repeat units, which contains many reactive -Si-H groups and hydrophobic -Si-CH_3 groups, while the former groups are also reactive with hydroxyl groups. Figure 3-9 shows the chemical structure of PMHS. Due to their hydrophobic nature, these additives facilitate lipophilic interactions and can specifically bind to the hydrophobic endogenous chemicals present in sebaceous latent fingermarks, including squalene, wax esters, triglycerides, phospholipids, glycerides, cholesterol, cholesterol esters and free fatty acids.[119] In addition, mica has been proven to be the most positively charged triboelectric material according to the triboelectric series of thirty inorganic nonmetal materials due to the even distribution of K^+ cations.[120-122] As fingermark residues contain natural secretions that are negatively charged,[123-125] the electrostatic interactions between the positively charged mica and the negatively charged fingermark residues would also be helpful for fingermark development.

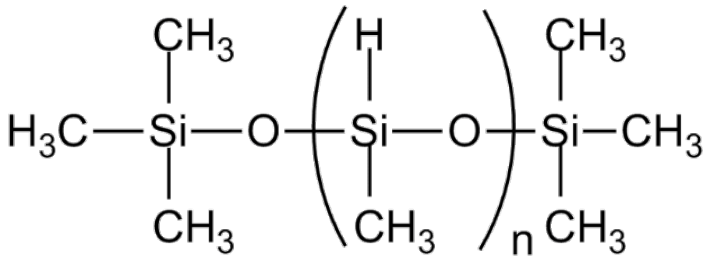


Figure 3-9. Chemical structure of PMHS with hydrophobic -Si-CH_3 groups.

3.3.3 Sensitivity and stability of fire extinguisher dry powder for latent fingermarks

The sensitivity of fire extinguisher dry powder to detect various fingermarks containing different amounts of residue deposited was investigated. This is due to the fact that in real-life casework scenarios, it is very common to encounter a high degree of variability in the quality of fingermark deposition. Figure 3-10 and 3-11 demonstrates that bright fingermark images with clear ridge details were observed for fingermarks developed with Eversafe fire extinguisher dry powder. Even with the weakest deposits, the resulting images displayed visible ridge detail with limited background staining, proving that fire extinguisher dry powders outweighed the traditional fingerprint powders in terms of detection sensitivity.

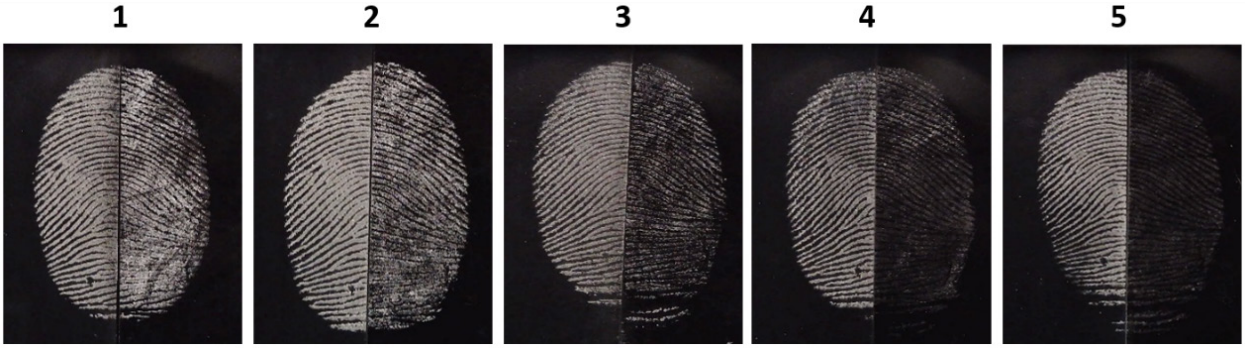


Figure 3-10. Comparison of five depletion split fingermark samples developed with Eversafe ABC powder (left) and white latent fingerprint powder (right) on glass slides.

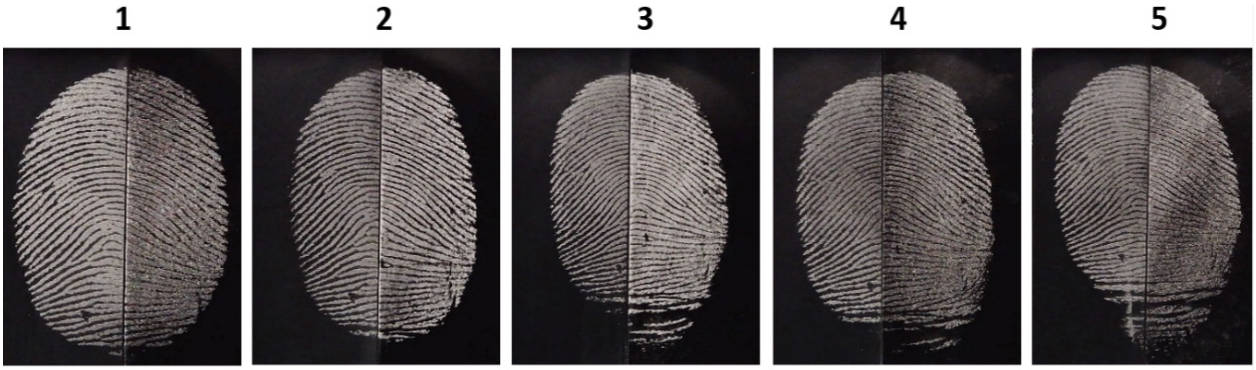


Figure 3-11. Comparison of five depletion split fingermark samples developed with Eversafe ABC powder (left) and white magnetic fingerprint powder (right) on glass slides.

In the stability study (i.e., time study), all the deposited natural and sebaceous fingermarks were stored in the dark and aged for nine ageing periods before visualizing with one of the six development methods. In previous studies, the new and novel spraying technique for applying fire extinguisher dry powder was only tested on fresh and sebaceous-rich fingermarks, overlooking its application on aged and natural impressions which are the representatives of actual operational

casework.[102-105] Figure 3-12 displays the results of the ageing study at various time intervals. All development methods were more effective for the detection of fresher fingermarks (with a mean grade between 3 to 4) and there was an observed reduction in the quality of the developed fingermarks with increased ageing periods. This was anticipated as older fingermarks contained smaller quantities of residues compared to fresher ones. Surprisingly, Eversafe fire extinguisher dry powder performed the best for all the age intervals; the mean grades of aged fingermarks developed were in the range of 2.8 to 3.7 and were consistently higher than the other development methods for any storage time interval. Even for old fingermarks up to 12 weeks old, over 88% of the fingermark samples had a grade of 3 or 4 and were categorized as "useful" for potential identification, proving that the fire extinguisher dry powders were hydrophobic enough to adhere strongly to the hydrophobic constituents which made up the bulk of aged latent fingermarks. The results demonstrated the feasibility of using fire extinguisher dry powder to develop latent fingermarks operationally.

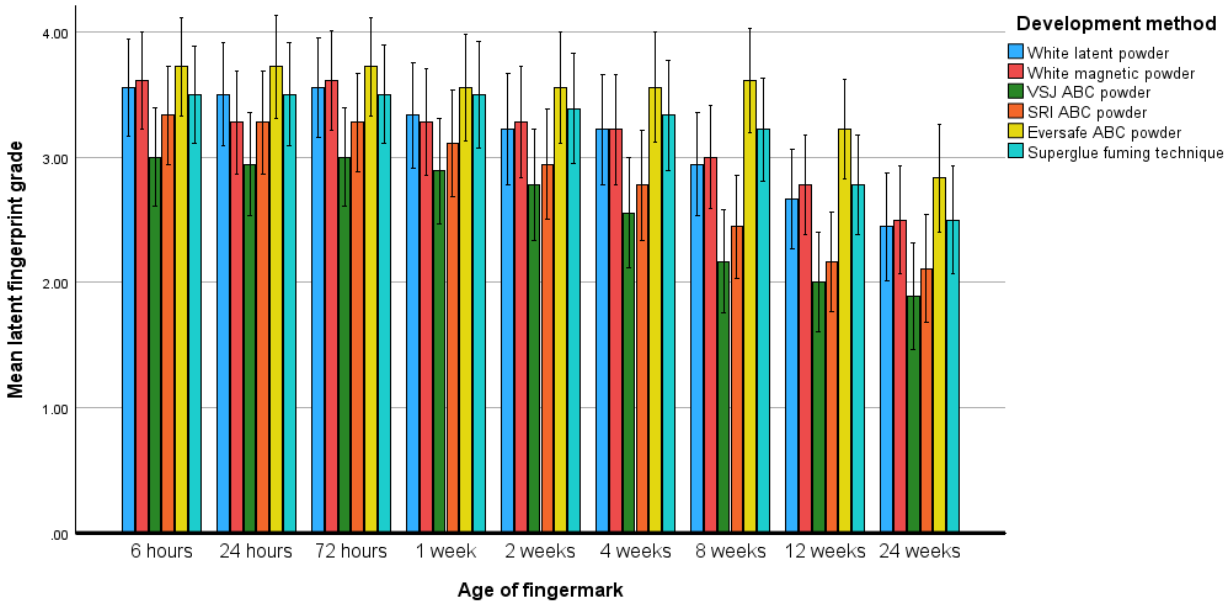


Figure 3-12. Comparison of the mean latent fingermark grades developed using six development methods for aged fingerprints.

The ability to reveal the detailed features of latent fingermarks is crucial for individual identification, which can also be used to quantify the sensitivity of latent fingermark detection. The latent fingermarks developed with Eversafe fire extinguisher dry powder are shown in Figure 3-13. The clear and well-resolved ridge patterns allowed clear visualization of level 1 (whorl ridge pattern), level 2 (information on ridge ending, bifurcation and spur) and level 3 (location and size of sweat pores and scar) information.

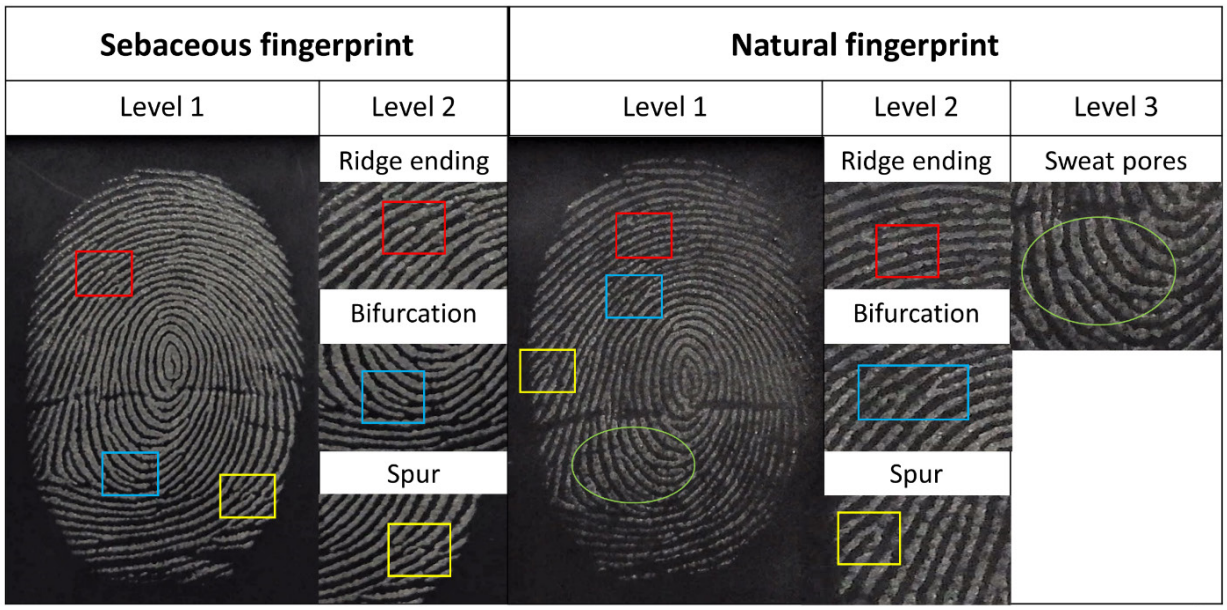


Figure 3-13. Specific details of fingerprint images using Eversafe fire extinguisher dry powder.

To discuss the contrast of fingermarks developed by different methods, the grayscale value (G) was extracted from up to down along the selected yellow line in a split fingermark using software Image J. The fingermark images and corresponding grayscale distribution diagrams are shown in Figure 3-14, showing the highest and lowest grayscale values of ten consecutive groups of papillary ridges and furrows. By looking at the grayscale distribution diagrams, fingermarks developed by Eversafe fire extinguisher dry powder had consistent pixel intensities at the ten consecutive groups of papillary ridges and furrows, providing insights into the level of detail or sharpness. These results strongly proved the high resolution and high detecting quality of fire extinguisher dry powders in fingermark development.

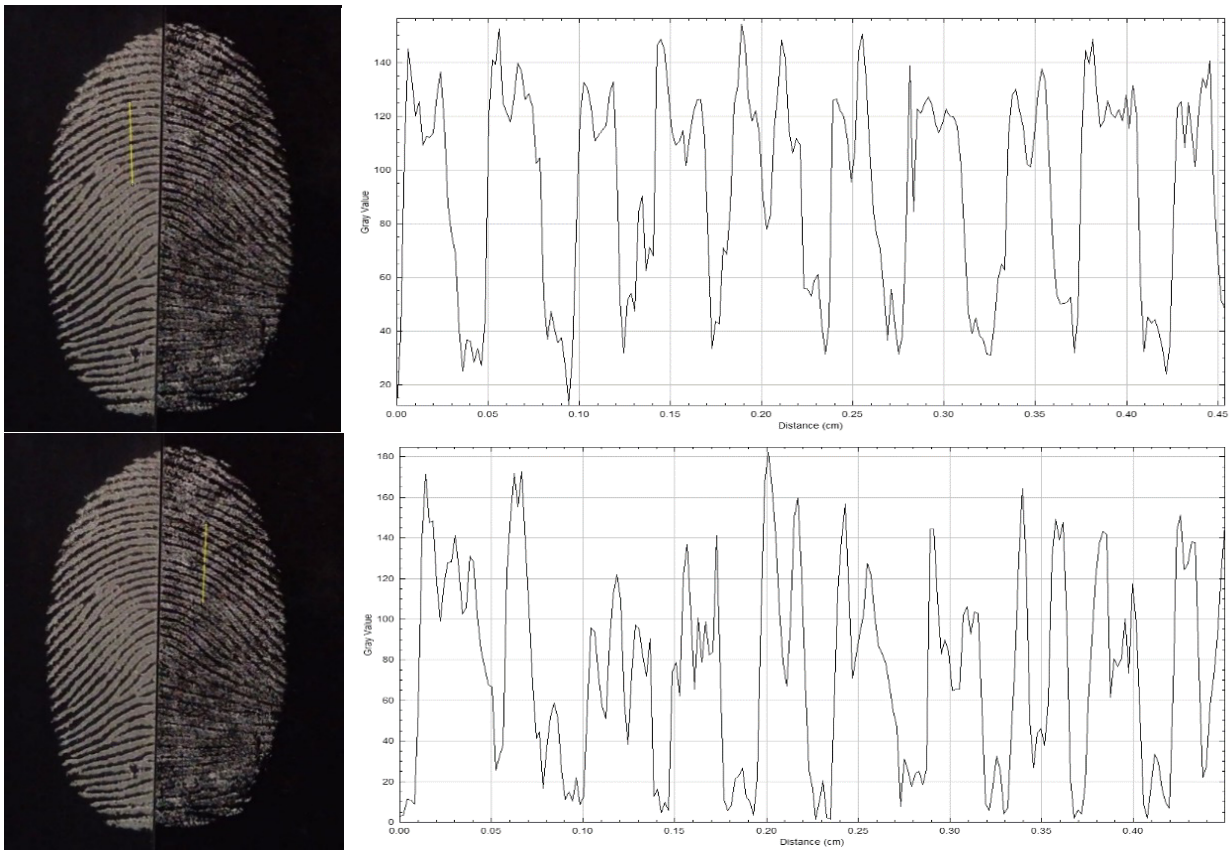


Figure 3-14. Latent fingerprints developed with Eversafe ABC powder (top) and white magnetic fingerprint powder (bottom) with the corresponding grayscale distribution diagram of the specific yellow line in each image.

Spraying the entire crime scene with a low cost of an ABC-type dry chemical fire extinguisher does not require specialized equipment and skills and can be done in a few seconds without any search examination, which eliminates the laborious task of powdering the entire scene using a brush. As spraying of fire extinguisher dry powder is cheap, timely, efficient and less labor-intensive, while exhibiting superiority performance in fingermark development, the use of fire extinguisher dry powder as a novel "*in situ*" development powder in a typical forensic operational laboratory is feasible. As the focus of the study was to investigate selectivity, sensitivity and

stability of fire extinguisher dry powder, it was completed with direct comparisons to two traditional fingerprint powders and cyanoacrylate fuming, and no subsequent enhancement techniques were applied. Although further enhancement may improve the fingermark quality in some cases, it was beyond the scope of this study, and future work may be required to explore if subsequent enhancement techniques can be conducted after spraying the crime scene to increase the quality and clarity of fingermarks being developed. In addition, further research is required into the impact on other types of evidence such as DNA, as well as the influence of various storage conditions to establish the superiority and applicability of fire extinguisher dry powders in routine forensic casework.

3.4 Conclusions

To save time and minimize adverse health effects such as cancer, asthma and other respiratory diseases to forensic scientists, criminologists and other investigators when handling large crime scenes and bulky evidence items with extensive surface areas, there is an urgent need for a fast, alternative on-site method for developing high-quality latent fingermarks. In this study, we have successfully demonstrated the selectivity, sensitivity and stability of fire extinguisher dry powders that can be successfully applied to develop fresh as well as aged latent fingermarks on different glass substrates. The results depicted that fire extinguisher dry powders offered a selective

interaction with the moisture and oily components in the fingermark residues, and thus offer clear and sharp images of fingermark ridges, even for latent fingermarks with low deposition quality. EDX analysis also confirmed the presence of PMHS with hydrophobic -Si-CH_3 groups, as well as the positively charged mica, which could attribute to the selectivity of fire extinguisher dry powders to fingermark residues. The performance of different brands of fire extinguisher may be different as there could be some variations in the concentrations of the elements according to manufacturers. These powders had been proven to outweigh traditional fingerprint powders and cyanoacrylate fuming due to four reasons: (1) cheap and commercially available, (2) safe as particles with average sizes of around 15 μm are large enough to be effectively captured using face masks, (3) high selectivity to fingermarks with weak deposits, such as old fingermarks up to 12 weeks old and (4) quick and simple by spraying the entire crime scene. The findings of this study could assist forensic scientists, criminologists and other investigators in making an informed decision when choosing a detection technique for '*in situ*' latent fingermarks.

Chapter 4: Chemical imaging of latent fingermarks developed with fire extinguisher dry powder using MALDI-MSI

4.1 Introduction

Currently, fingermark identification is carried out for the purpose of matching by developing the latent fingermarks at the crime scene and comparing the visible ridge patterns with those stored in national databases.[126] A match cannot be obtained if the fingermarks are faint, smudged, distorted, partial in nature or absent in record. Subjectivity in fingermark comparison must also be addressed as faulty fingermark evidence could lead to wrongful convictions when they are brought into a court of law.[127] As a result, it is necessary to adopt robust chemical techniques that explore beyond the morphology. The position, orientation and chemical composition should be investigated to obtain information about the donor of the fingermarks and link the donor to the suspect through crime scene reconstruction, as shown in Figure 4-1.[128]

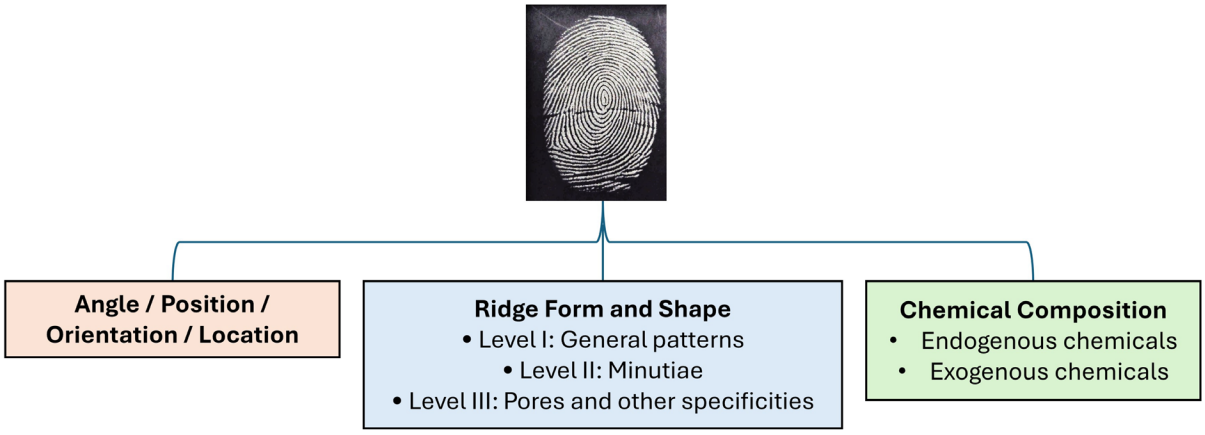


Figure 4-1. Information obtained from a latent fingermark.[128]

According to the Locard's exchange principle that every contact leaves a trace, fingermarks result as a transfer of sweat and chemical compounds from the papillary ridges of fingertips to various surfaces during contact.[129] Latent fingermarks, the most common type of fingermarks encountered in crime scenes, have complex composition, and are mainly composed of a mixture of numerous compounds originating from three sources: (1) the epidermis, (2) the apocrine, sebaceous and eccrine glands within the dermis and (3) semi-endogenous and exogenous contaminants.[130-133] Table 4-1 provides a breakdown of the composition of the endogenous secretions from the epidermis, apocrine, sebaceous and eccrine glands.[130-133] Examples include fatty acids, amino acids, diacylglycerol (DAGs), wax esters (WEs), squalene, triacylglycerols (TAGs), vitamins and water.

Chemical profile, i.e., the presence of endogenous compounds (i.e., naturally occurring compounds originated from the human body), semi-endogenous compounds (i.e., compounds result from the inhalation or ingestion of drugs and medication, as well as food and drinks) and exogenous compounds (i.e., contaminants from external sources due to a prior contact such as cosmetics, hair, condom lubricants and gunshot residues) derived from the latent fingermarks collected in the crime scenes can enhance the evidentiary value of fingermarks when combined with ridge patterns.

Table 4-1. Summary of the endogenous residues in latent fingermarks and their sources.[130-133]

Source	Organic constituents	Inorganic constituents
Epidermis	Fatty acids; Glycerides; Proteins (e.g., cathepsin D, keratins 1 and 10); Sterols (e.g., cholesterol); Sterol esters (e.g., cholesterol ester)	
Apocrine glands (Apocrine sweat)	Androgenic steroids; Carbohydrates (e.g., glycogen); Carboxylic acids; Proteins; Sterols (e.g., cholesterol)	Ammonia; Iron; Water
Sebaceous gland (Sebum)	Fatty acids (e.g., palmitic, palmitoleic, oleic, stearic, myristic acids); Fatty acid alkyl esters (e.g., palmitic acid methyl ester, stearic acid methyl ester). Glycerides (e.g., mono-, di- and triglycerides); Hydrocarbons (saturated and unsaturated); Squalene; Squalene degradation products (e.g., squalene epoxides, squalene hydroperoxides); Sterols (e.g., cholesterol); Sterol esters (e.g., cholesterol esters); Wax esters (e.g., myristyl myristate, palmityl palmitoleate, stearyl palmitate)	
Eccrine gland (Eccrine sweat)	Amino acids (e.g., serine, glycine, ornithine, alanine, aspartic acid); Creatine; Creatinine; Enzymes (e.g., esterases, proteolytic enzymes); Glucose and other reducing sugars; Glycogen; Lactic acid and lactate; Peptides (e.g., dermicidin, cathelicidin LL-37); Phenol; Proteins (e.g., albumin, cathepsin D, immunoglobulins (IgG, IgA, IgD, IgE), keratins 1 and 10); Pyruvic acid and pyruvate; Urea; Uric acid; Vitamins (e.g., ascorbic acid, choline, folic acid, niacin, riboflavin)	Ammonia; Bicarbonate; Bromide; Chloride; Fluoride; Iodide; Metal ions – major (e.g., calcium, iron, potassium, sodium) and trace (e.g., cobalt, copper, lead, magnesium, zinc); Phosphates; Sulphates; Sulphide; Water (98%)

Over the past years, many analytical techniques have been proposed to investigate the chemical composition of fingermarks. The technique is aimed to be specific, quantitative and reproducible. More importantly, it should be readily available to forensic laboratories, and it must be compatible with the forensic routine practice.

Thin-layer chromatography (TLC) was first employed in 1986 to study the chemical transformations of chemical substances of perspiration found in latent fingermark residues as a function of time and environment.[134] It is infeasible to use TLC on real crime scene samples due to its poor resolution, low sensitivity and low accuracy. It is non-specific, thus can only find out the various classes of chemicals being present and cannot provide great detail about the composition of the fingermark.[134-135]

Fourier Transform Infrared (FTIR) spectroscopy is another common technique to investigate the fingermark chemistry, such as detecting fingermarks on different substrates, determining the characteristics of donors such as age and gender, and estimating the time since deposition.[136-139] Compared to TLC, FTIR spectroscopy can give a more comprehensive understanding of the ridge patterns and characteristics of the latent fingermarks by obtaining the distribution of specific functional groups and chemical bonds in the residues (i.e., the lipid components), and at the same

time, producing a chemical map by chemically imaging the residues.[136-139] Although FTIR spectroscopy is a non-destructive technique and requires no sample preparation, it has poor chemical specificity and limits analysis to fingermarks deposited on infrared-transparent substrates that are relatively rare and unrealistic in terms of real crime scene scenarios.[136-139]

Conventional extraction-based chromatography methods such as gas chromatography mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LC-MS) have been previously shown that sebaceous and eccrine secretions in latent fingermarks can be extracted into a solvent and analyzed.[140-144] GC-MS targets more volatile and thermally stable compounds (e.g., small lipids and amino acids), [140-142] while ultra-performance LC-MS targets larger molecules (e.g., glycerides, peptides and proteins) and can elucidate the structure of the target compounds and aging products.[143-144] However, sample preparation and extraction, as well as the development and optimization of the techniques, is complicated and laborious. In the forensic aspect, these techniques are undesirable to real crime scene scenarios as they destroy the whole fingermark evidence through extraction in order to analyze its composition. Furthermore, these destructive techniques analyze the total extract of both the fingermarks and surface chemicals, thus cannot distinguish the two. To overcome these issues, researchers have switched to find a more reliable and non-destructive method for analyzing the chemical composition of latent fingermarks.

MALDI-MSI is a powerful analytical tool that provides a spatially resolved chemical profile of a sample, as determined by the mass-to-charge ratio (*m/z*) of compounds being present.[145] The first reported application of MALDI-MSI to the analysis of fingermarks was the imaging of endogenous lipids from sebaceous and eccrine fingermarks conducted by Wolstenholme et al. in 2009.[146] The imaging of fingermark samples was performed with the chemical profile of oleic acid and its degraded products which enabled the distinction of the different fresh and aged fingermarks.[21] In the recent years, this technique has been applied to the analysis of latent fingermarks by providing a visual image of ridge details and a chemical profile of the spatial distribution of the chemical species (i.e., the endogenous and exogenous compounds in a fingermark) in only one single analysis.[147] The abundance of these compounds on the spectra obtained can then be utilized to potentially reconstruct an individual's lifestyle and recent activities[148-149] and establish personal information about the individual such as the gender[150-151] and diet.[152-153] Ferguson et al. determined the sex of a fingermark donor with a 85% accuracy by comparing protein and peptidyl profiles.[150] There are also different studies in exploring a new MALDI matrix to enhance the effect of the laser in initiating the desorption/ionisation process, which can also serve a role as fingermark development powder.[154-155] Francese et al. reported the dual role of a naturally occurring substance, curcumin, as a fingermark development powder and a versatile matrix to detect lipids, pharmaceuticals and drugs.[154] Sundar and Rowell detected drugs of abuse (cocaine and methadone) and therapeutic drugs (aspirin, caffeine and paracetamol) in lifted cyanoacrylate-developed latent

fingermarks.[156] As a result, such details can be used to narrow down the pool of suspects and differentiate fingermarks of different individuals, especially in cases where no match is found in a fingerprint database search. This technique is also non-destructive as the matrix can be washed away without damaging the pattern,[146] which is important in a court of law.

In the previous chapter, a new and novel fingermark detection method - fire extinguisher dry powder was demonstrated to be quick, simple, cost-effective, sensitive and selective to the moisture and oily components in the fingermark residues, thus offering clear and sharp images of fingermark ridges. In circumstances when a match cannot be obtained for fingermarks which are smudged, distorted, partial in nature or absent in record, direct analysis of the chemicals embedded in the fingermarks should be performed to build a profile of a suspect. Therefore, the use of fire extinguisher dry powder must be compatible with MALDI-MSI to allow this new fingermark detection method to be incorporated into the field of forensic science. If fire extinguisher dry powders were demonstrated to enhance the level of retrievable physical characteristics (as in Chapter 3) and to be compatible with MALDI-MSI (as in Chapter 4), the use of fire extinguisher dry powder can become the best alternative to conventional fingerprint powders and other fingermark detection methods, especially when processing large crime scenes and bulky evidence items with large surface areas. This type of work has not yet been reported and could be highly

useful to help forensic scientists, criminologists and other crime scene investigators utilizing the full potential of latent fingermarks with a new *in-situ* development method.

Objectives of the research study:

a) To develop and optimize a method for MS profiling and imaging to detect endogenous and exogenous compounds on latent fingermarks that have been developed with fire extinguisher dry powders;
b) To determine whether the spraying process of fire extinguisher dry powders affects the subsequent MALDI-MSI for the profiling of endogenous and exogenous chemicals and imaging of friction ridges (i.e., compatibility with chemical imaging of latent fingerprints).

4.2 Experimental section

4.2.1 Study design

This study was completed in two parts to conduct a full evaluation of the use of fire extinguisher dry powder to detect latent fingermarks using MSI (Figure 4-2). The first part focused on the optimization of MALDI-MS protocol for fingermark analysis. Three parameters such as matrix selection, matrix concentration and number of passes of matrix were tested to establish a protocol that can produce high quality and reproducible spectra. The second part was a comparative study to directly analyze endogenous and exogenous compounds (due to prior handling of a condom, an aspirin pill or personal and household products) embedded in latent fingermarks with and without prior development with fire extinguisher dry powders. Distributions of these compounds were also imaged without disturbing the patterns. Capability of separating two overlapping fingermarks developed with fire extinguisher dry powders were also investigated.

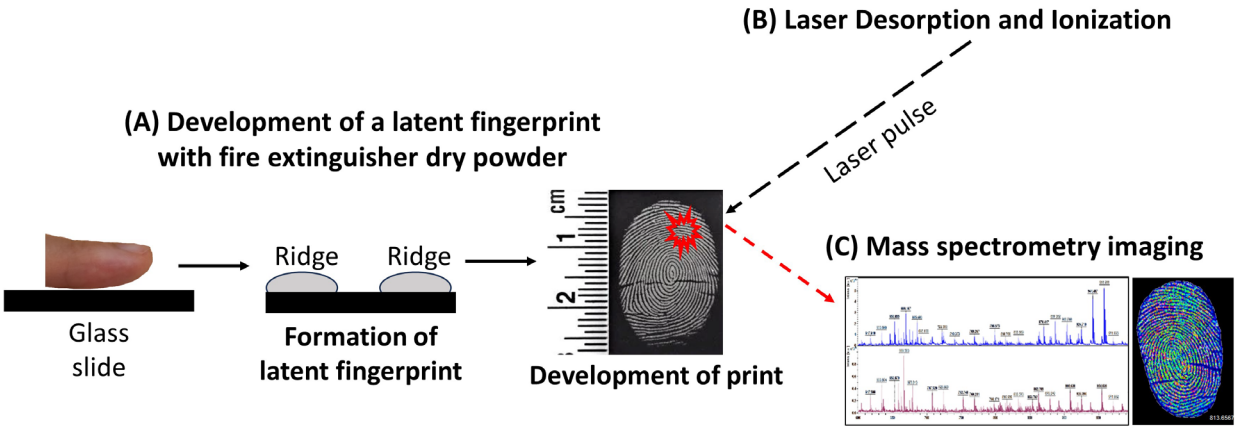


Figure 4-2. Schematic diagram showing the development of a latent fingermark using fire extinguisher dry powders and chemical analysis using MALDI-MSI.

4.2.2 Chemicals and materials

MALDI matrices DHB and CHCA were purchased from Aldrich (St. Louis, MO, USA). HPLC grade acetone and methanol were purchased from Anaqua Chemical Supply (Houston, TX, USA). Conductive indium tin oxide (ITO) coating glass slides were supplied by Bruker Daltonics. Eversafe fire extinguisher was chosen as it was proven in Chapter 3 to be the best in developing latent fingermarks by exhibiting the highest mean grade (mean grade of 4) among the development methods on clear float glass. Condoms from two widely available brands namely *Trojan* (*ENZ* Armor Spermicidal Lubricated Condoms) and *Okamoto* (B-7 Beyond Seven Plus) were purchased from supermarkets. Aspirin pills from a brand named *Bayer* was purchased through a commercial website.

4.2.3 Latent fingermark deposition and preparation

Sebaceous fingermarks were deposited on the conductive glass slides by six healthy individuals, three males and three females with their age ranging from 20 to 50 years old. Donors were asked to thoroughly wash, rinse and dry the hands to remove any greasy matter. After rubbing the fingers on oily regions such as the nose, cheeks or forehead, fingermarks were deposited across two adjacent glass slides with moderate force for three seconds as split marks. One half of the fingermark was developed with fire extinguisher dry powders and another half was not.

In the preparation of the exogenous compound-contaminated latent fingermarks, a condom or an aspirin pill were touched or handled with the sebum-rich fingers as if being used or consumed, mimicking a real usage prior to fingermark deposition. In the preparation of the overlapping latent fingermarks (i.e., a vertical fingermark at the bottom overlapping with a horizontal fingermark on the top) containing different chemical compounds, a donor was asked to handle a condom as if being consumed prior to deposition of the vertical fingermark, while another donor was asked to deposit a sebaceous fingermark on the top. As with traditional application, Eversafe fire extinguisher dry powder was slowly sprayed from each cylinder with the procedures stated in Chapter 3 after fingermark deposition.

4.2.4 Matrix application

All fingermark samples were coated with CHCA matrix prepared at a concentration of 5 mg/mL in a 70:30 ACN/0.1% TFA using a TM-Sprayer (HTX Technologies, LLC). 4 coatings were deposited without any dry time between passes. The flow rate, spray nozzle velocity, spray nozzle temperature, spacing between tracks and nitrogen pressure were set at 0.1 mL/min, 1200 mm/min, 75°C, 3 mm and 10 psi, respectively. Conventional MALDI matrices (CHCA and DHB), CHCA concentrations of 7.5 mg/mL and 10 mg/mL, as well as 2 and 3 coatings were also tested for the optimization of protocol.

4.2.5 MALDI-MS and MSI analysis

An UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker, Billerica, MA, USA) equipped with a 355 nm smartbeam-II laser was used for latent fingermark analysis. The glass slides were mounted via the MTP Slide Adapter II (Bruker Daltonic, Germany). Experiments were performed in positive ion and reflectron mode, and pre-calibrated for accurate masses. The spectra were obtained with a *m/z* range of 100-1000 Da with instrument parameters such as ion source voltage 1, ion source voltage 2, lens voltage, reflector voltage 1 and reflector voltage 2 set to 20.00 kV, 17.95 kV, 8.50 kV, 21.10 kV and 10.85 kV respectively. The ion pulse extraction was set to 140 ns, and each shot included 1000 laser pulses. For each fingermark sample, signals from at least 5 random positions (1000 laser pulse per one position) on the sample spot were acquired manually. The mass spectra were accumulated and saved as the final spectra when its absolute intensity of the top peak reached $1x10^4$. Control MS spectra were also obtained from the surface of glass slides with and without donor fingermarks that were not treated with fire extinguisher dry powders. The MSI data acquisition was performed using a spatial resolution of 150 μm and collecting a total of 500 laser shots per pixel by using the "medium" laser focus setting. The instrument was controlled by the FlexControl 3.4 and FlexAnalysis 3.4 program (Bruker, Billerica, MA, USA). All the chemical images were reconstructed and visualized using FlexImaging 4.0 (Bruker, Billerica, MA, USA).

Compound identification was achieved by matching the accurate mass measurements in the online databases (e.g., the LIPID MAPS database: www.lipidmaps.org/) and previously published results. Confirmation of chemical compounds was also performed by comparing accurate mass measurements to previously published results. The identity of some compounds was further confirmed by MS/MS measurements acquired in LIFT-TOF/TOF mode. Separate MS/MS experiments were also performed for selected precursor ions.

4.3 Results and discussion

4.3.1 Optimization of MALDI-MS protocol for direct analysis of latent fingermarks

Three parameters such as matrix selection, matrix concentration and number of passes of matrix were tested to establish a protocol that can produce high quality and reproducible spectra. Comparison of split fingermarks was performed for a direct comparison of the parameters from the same fingermark deposition (i.e., with the same chemical composition and quantity of chemicals). MS signals, i.e., the intensity and number of detected compounds, were evaluated. No fingermark development was performed in this section.

Matrix selection in positive and negative modes

Donors were asked to deposit sebaceous latent fingermarks, and one half of a fingermark was sprayed with conventional MALDI matrix DHB and another half was sprayed with CHCA. Spectra were acquired in five random regions to observe the behavior of endogenous and exogenous compounds.

Figure 4-3 and Figure 4-4 show the representative MALDI-MS spectra in positive mode in *m/z* 100-400 and 400-1000 respectively. Good spectra were obtained for small analytes such as fatty acids on the latent fingerprints for both matrices. Some of the peaks in the mass spectra of the latent fingermarks were similar to the ones obtained when using pure CHCA and DHB matrices (control MS spectra that were obtained from the surface of glass slides without donor fingermarks).[157] Examples of some CHCA and DHB matrix ions detected on the latent fingermarks are shown in Table 4-2.

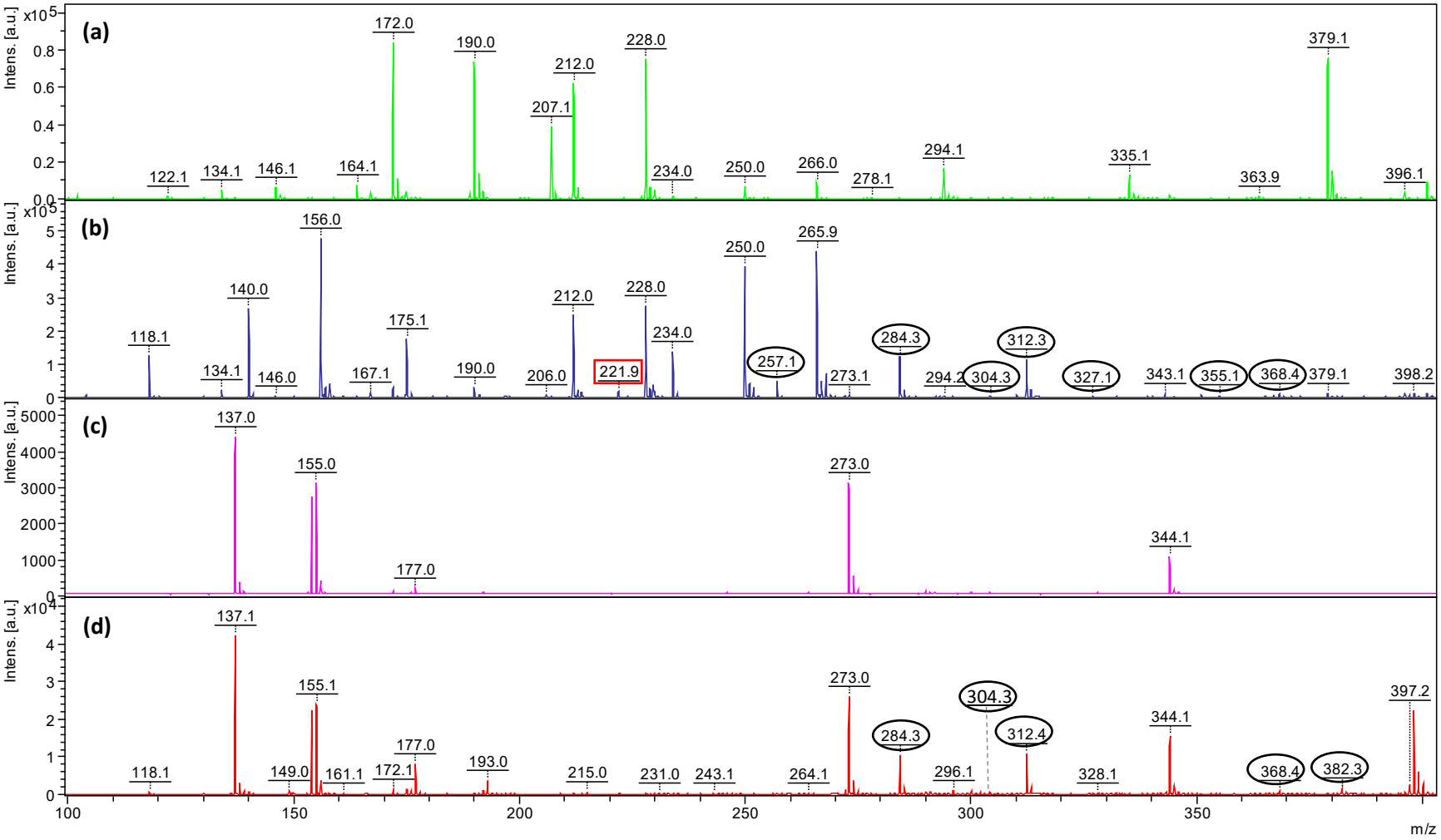


Figure 4-3. MALDI-MS analysis in positive mode between *m/z* 100 and 400. (a) Mass spectrum of blank CHCA matrix. (b) Mass spectrum of a half fingermark sprayed with CHCA matrix. (c) Mass spectrum of blank DHB matrix. (d) Mass spectrum of a half fingermark sprayed with DHB matrix.

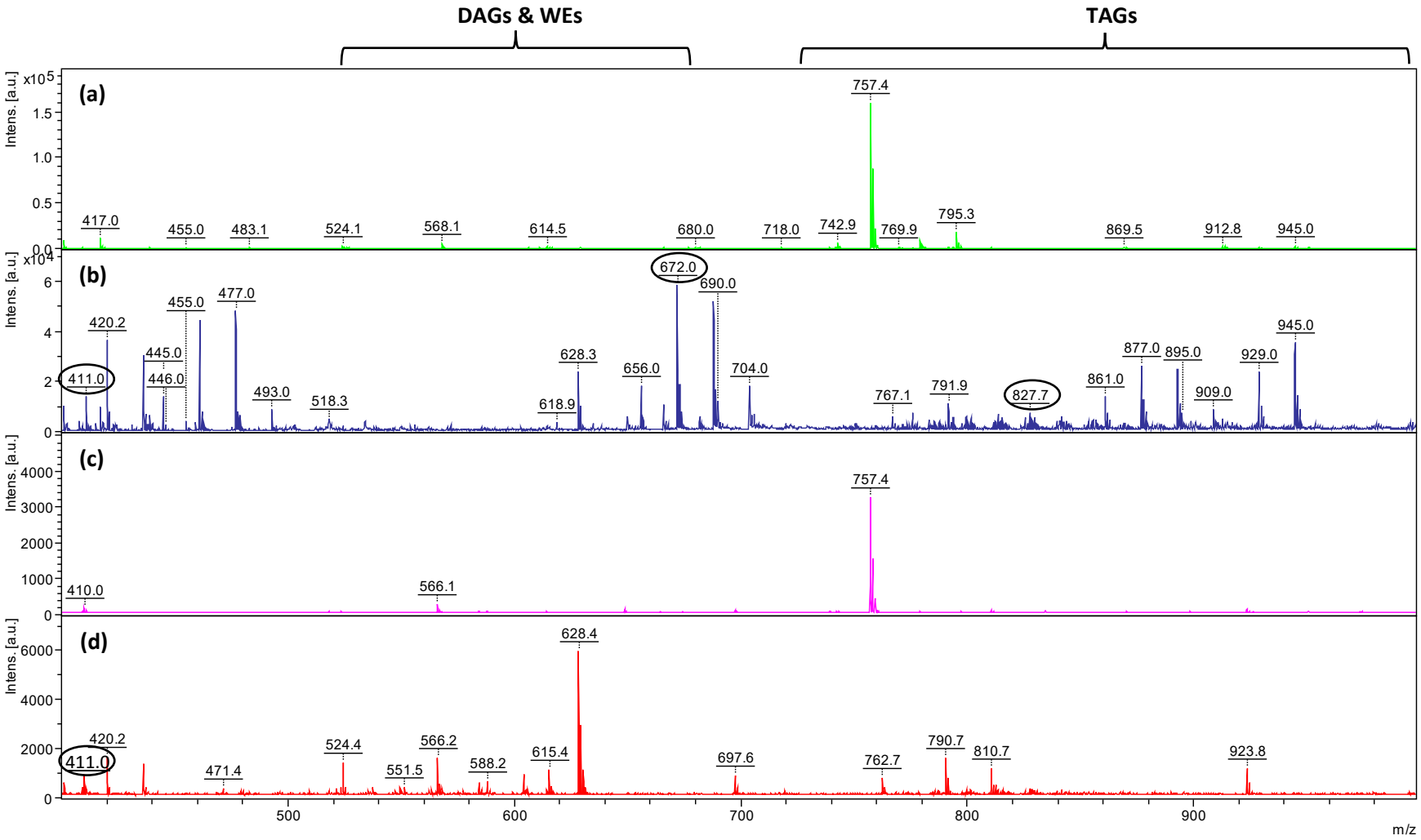


Figure 4-4. MALDI-MS analysis in positive mode between *m/z* 400 and 1000. (a) Mass spectrum of blank CHCA matrix. (b) Mass spectrum of a half fingermark sprayed with CHCA matrix. (c) Mass spectrum of blank DHB matrix. (d) Mass spectrum of a half fingermark sprayed with DHB matrix.

Table 4-2. Overview of all identified CHCA and DHB ions detected on the latent fingermarks in positive ion mode in *m/z* 100-1000.[157]

CHCA matrix			
Cluster composition	**Sum formula**	**Theoretical *m/z***	**Measured *m/z***
$[M+H-CO_2]^+$	$C_9H_8NO^+$	146.060	146.036
$[M+H]^+$	$C_{10}H_8NO_3^+$	190.050	190.023
$[M+Na]^+$	$C_{10}H_7NO_3Na^+$	212.032	212.016
$[M+K]^+$	$C_{10}H_7NO_3K^+$	228.006	228.004
$[M-H+2Na]^+$	$C_{10}H_6NO_3Na_2^+$	234.014	233.994
$[M+Na+K-H]^+$	$C_{10}H_6NO_3NaK^+$	249.988	249.971
$[M+2K-H]^+$	$C_{10}H_6NO_3K_2^+$	265.962	265.946
$[2M+H]^+$	$C_{20}H_{15}N_2O_6^+$	379.093	379.072
$[2M+Na]^+$	$C_{20}H_{14}N_2O_6Na^+$	401.074	401.052
$[2M+K]^+$	$C_{20}H_{14}N_2O_6K^+$	417.048	417.046
$[2M+2K]^+$	$C_{20}H_{13}N_2O_6K_2^+$	455.004	455.000
$[3M+Na+3K-3H]^+$	$C_{30}H_{18}N_3O_9NaK_3^+$	703.985	703.973
DHB matrix			
Cluster composition	**Sum formula**	**Theoretical *m/z***	**Measured *m/z***
$[M+H-H_2O]^+$	$C_7H_5O_3^+$	137.023	137.059
$[M+H]^+$	$C_7H_7O_4^+$	155.034	155.061
$[M+Na]^+$	$C_7H_6O_4Na^+$	177.016	177.035
$[2M+H-2H_2O]^+$	$C_{14}H_9O_6^+$	273.040	273.046
$[3M+H-3H_2O]^+$	$C_{21}H_{13}O_9^+$	409.055	409.076

Table 4-3 shows the tentative identifications of compounds detected by MALDI-MS in the latent fingermarks. All the assigned compounds were detected as the protonated form, $[M+H]^+$, except cholesterol as $[M-H2O+H]^+$ and sodium adducts, $[M+Na]^+$ in case of TAGs cluster ions. The endogenous compounds studied included amino acids, fatty acids, cholesterol, peptides, proteins and TAGs. The tentative identifications were made on the basis of *m/z* and the assignments made by previous studies which examined and detected chemicals components in latent fingermarks. Each of these components occurred at the same *m/z* value in the fingermarks sprayed with CHCA matrix as they did in the fingermarks sprayed with DHB matrix, indicating that they did not undergo any chemical changes during the matrix application. However, signal intensities for the species were higher in the mass spectra with CHCA matrix and this was more noticeable for higher *m/z* values.

In terms of accurate mass measurement, ions at *m/z* 284.3 matched the m/z value of cetrimonium ion,[162] while ions at *m/z* 304.3 and 332.3 correspond to n-alkyl dimethylbenzylammonium (DMA) ions (with general formula of $[C_6H_5CH_2N^+(CH_3)_2C_nH_{2n}+1)]$ having 12 and 14 CH_2 repetition units, respectively.[155,163,164] These two ions have been reported as exogenous antibacterial species originated from toiletry products, wipes, hand sanitizers and detergents, and are commonly found in latent fingermarks.[164] On the mass spectrum with CHCA as the matrix, the ion at *m/z* 340.4 is

also a contaminant and has previously been detected and/or identified in fingermarks as didecyldimethylammonium ion, DDDMA.[165] However, it was not present when DHB was used as the matrix. Ions at *m/z* 340.4 and 368.4 are also exogenous quaternary ammonium ions found in lotions, having formulas of $C_{23}H_{50}N^+$ and $C_{25}H_{54}N^+$, respectively.[165,166] The identities of the peaks such as *m/z* 221.9 could not be identified on the latent fingermarks. This is common as there are many natural compounds excreted in fingermark sweat that have not been identified. The peak at *m/z* 757.4 is an unknown peak coming from either the matrix or a MALDI target plate. In addition, numerous endogenous compounds such as cholesterol esters, DAGs, WEs and TAGs were tentatively identified in the high *m/z* region. However, the intensities of MS signals were much lower when DHB was used as the matrix.

Table 4-3. Compounds detected by MALDI-MS in latent fingermarks. All compounds were tentatively identified.

Matrix	Observed *m/z*	Tentative identification with references	
CHCA	257.1	Fatty acids	palmitic acid[158,159]
DHB	/		
CHCA	285.3		stearic acid[158-160]
DHB	285.3		
CHCA	312.3		arachidic acid[152]
DHB	312.4		
CHCA	327.1		heneicosanoic acid[159]
DHB	/		
CHCA	355.1		tricosanoic acid[159]
DHB	/		
CHCA	382.3		tetracosadienoic acid[160]
DHB	382.3		
CHCA	431.1		vitamin E[155]
DHB	/		
CHCA	411.0	Squalene	squalene[159,161]
DHB	411.0		
CHCA	284.3	Exogenous compounds	cetrimonium[162]
DHB	284.3		
CHCA	304.3		dimethylbenzylammonium ion, DBA ($12CH_2$)[155,163,164]
DHB	304.3		
CHCA	332.3		dimethylbenzylammonium ion, DBA ($14CH_2$)[164]
DHB	332.3		
CHCA	340.4		didecyldimethylammonium ion, DDDMA[165]
DHB	/		
CHCA	368.4		behentrimonium[166]
DHB	368.4		
CHCA	672.0	TAGs	cholesterol ester[163]
DHB	/		
CHCA	827.7		TAG (48:1)[167]
DHB	/		

To confirm whether the low signal intensities of DAGs, WEs and TAGs in Figure 4-4 using DHB as matrix were due to the individual donor samples, other donors were asked to deposit sebaceous latent fingermarks. Figure 4-5 shows the MALDI-MS spectra of fingermark deposited by another donor in positive mode in *m/z* 400-1000. Again, the signal intensities of DAGs, WEs and TAGs dropped to 1500 when DHB was the matrix. As a result, CHCA was chosen as the matrix for the remaining experiments.

Figure 4-6 shows the representative MALDI-MS spectra in negative mode using CHCA as the matrix. Chemical interference was observed in MALDI matrix spectra and may have been the main issue with the low detection rate of all the chemicals. As a result, positive mode was used for the remaining experiments.

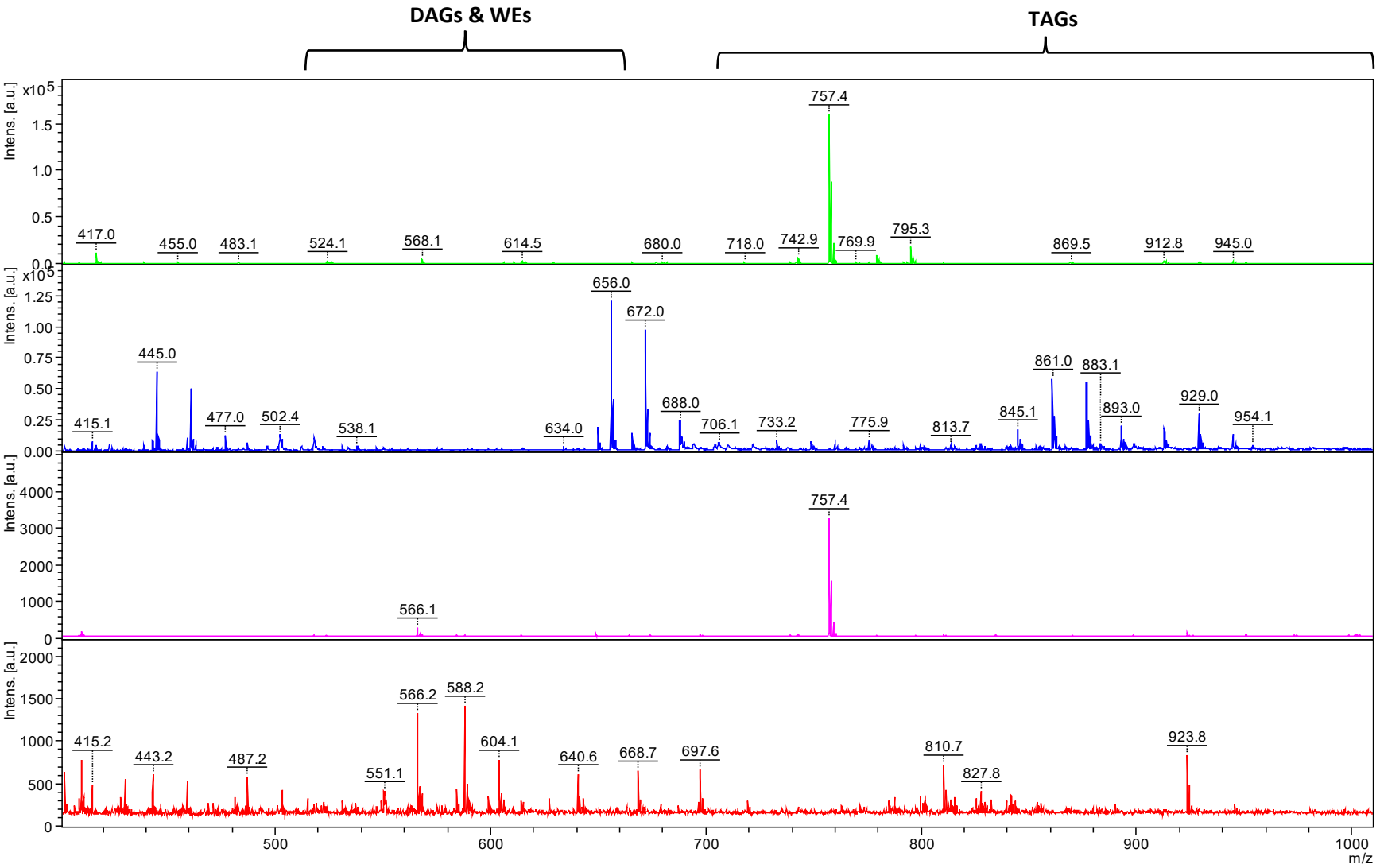


Figure 4-5. MALDI-MS analysis in positive mode between *m/z* 400 and 1000. (a) Mass spectrum of blank CHCA matrix. (b) Mass spectrum of a half fingermark sprayed with CHCA matrix. (c) Mass spectrum of blank DHB matrix. (d) Mass spectrum of a half fingermark sprayed with DHB matrix.

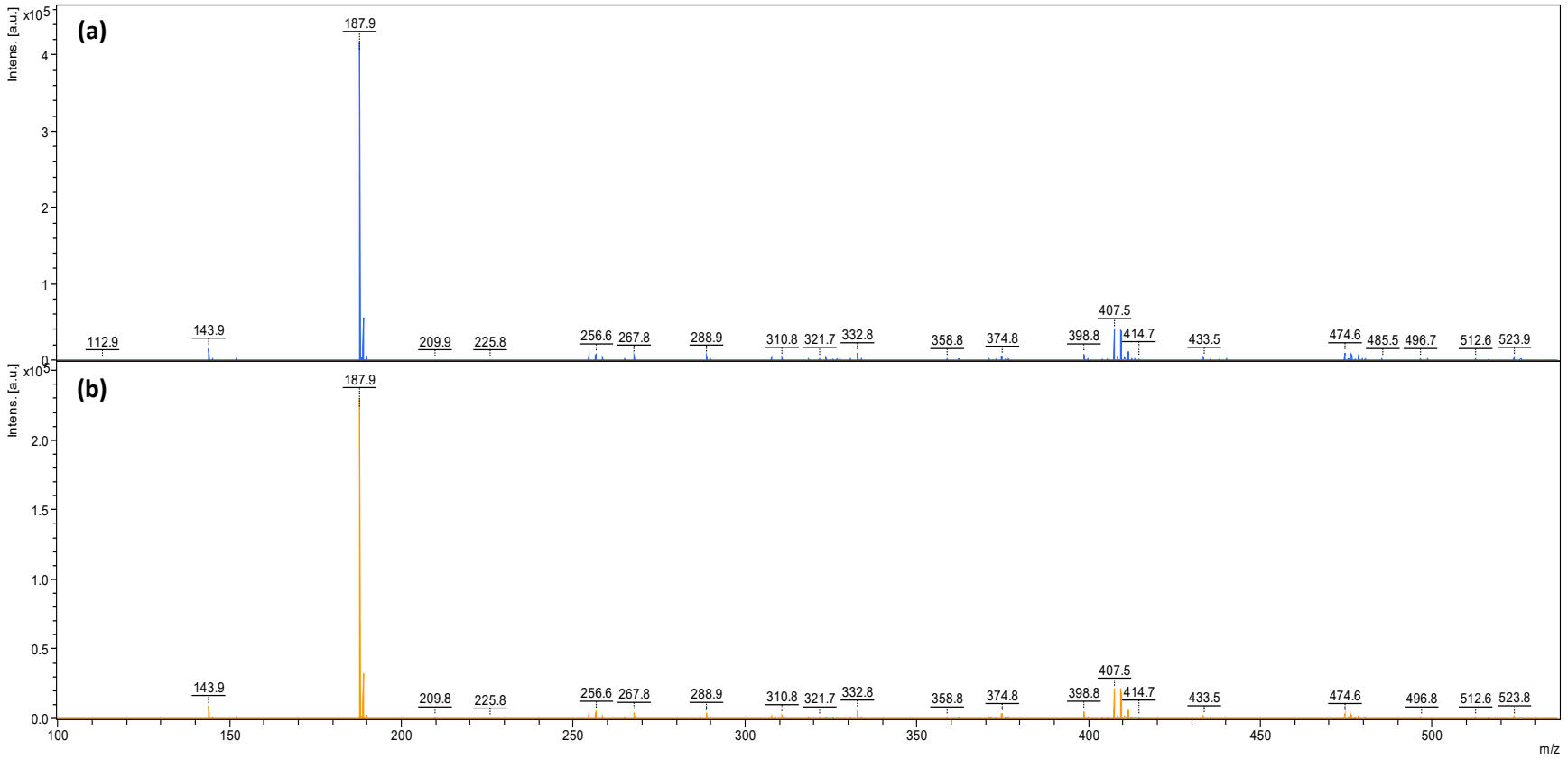


Figure 4-6. MALDI-MS analysis in negative mode between *m/z* 100 and 600. (a) Mass spectrum of blank CHCA matrix. (b) Mass spectrum of a latent fingermark sprayed with CHCA matrix.

Matrix concentration

CHCA concentrations of 5 mg/mL, 7.5 mg/mL and 10 mg/L were sprayed onto the same fingermark at various positions, while other parameters were kept constant. Figure 4-7 shows the MALDI-MS spectra from fingermark samples which were sprayed with different concentrations of CHCA matrix. It was found that the higher the matrix concentration was, the higher the peak intensities. It is also important to aware that the higher the matrix concentration, excess desorbed material may have a greater chance to clog the electrospray emitter or the ion transfer tube, which further requires the mass spectrometer to be cleaned frequently. As 5 mg/mL of CHCA can ionize the molecules present in the fingermark sufficiently, it was chosen for the remaining experiments.

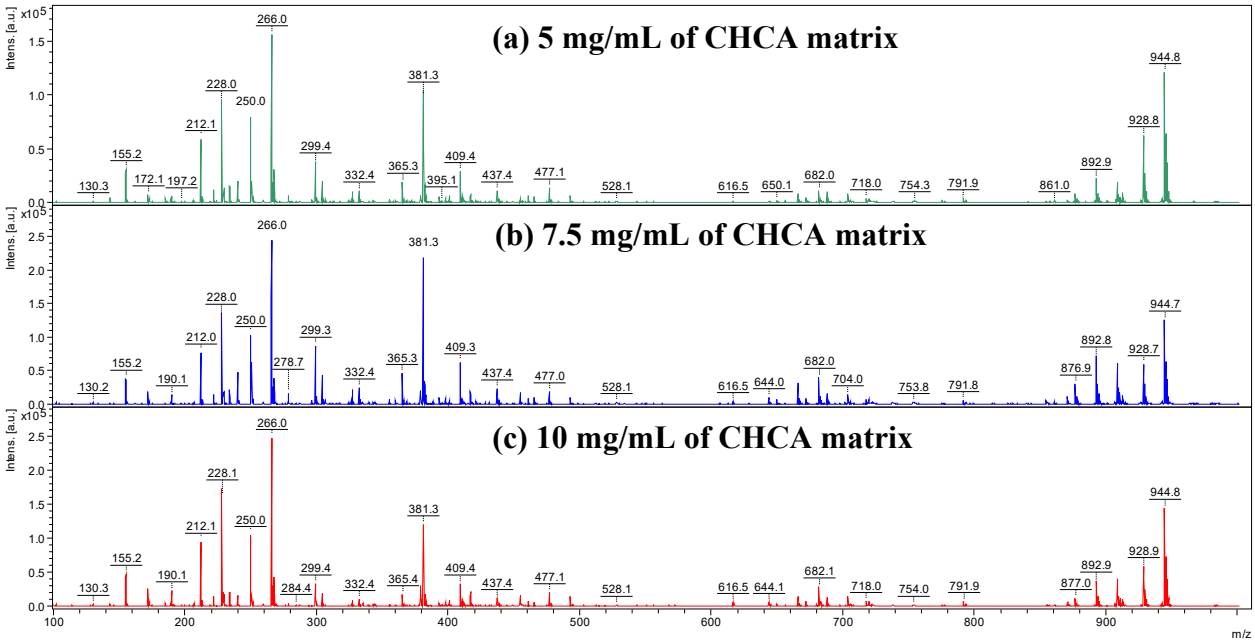


Figure 4-7. MALDI-MS analysis in positive mode for fingermark samples which were sprayed with different concentrations of CHCA matrix (a) 5 mg/mL, (b) 7.5 mg/mL and (c) 10 mg/L.

Number of passes of matrix

2 passes, 3 passes and 4 passes of CHCA matrices were sprayed onto the same fingermark at various positions, while other parameters were kept constant. Figure 4-8 shows the MALDI-MS spectra from fingermark samples which were sprayed with different passes of CHCA matrix. Although 2 passes of CHCA matrix was sufficient to produce a nice mass spectrum, there was a significant difference in signal intensities observed moving from 2 to 3 passes of matrix. For the mass spectrum with 4 passes, certain low abundance chemicals, such as the one at *m/z* 130.3 appeared, while excessive background peaks coming from the matrix were not taken place. As a result, 4 passes of CHCA matrix were chosen for the remaining experiments.

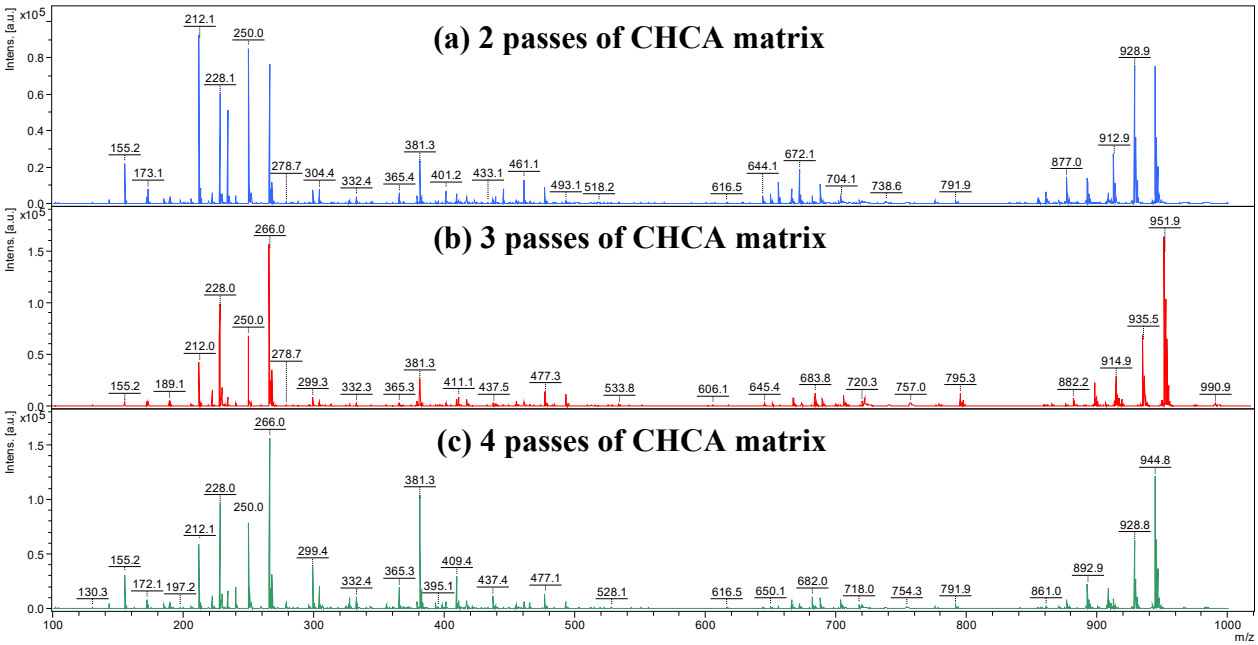


Figure 4-8. MALDI-MS analysis in positive mode for fingermark samples which were sprayed with different passes of CHCA matrix (a) 2 passes, (b) 3 passes and (c) 4 passes.

In conclusion, the best results were achieved in the positive mode when using 4 passes of CHCA at a concentration of 5 mg/mL in 70:30ACN/ 0.1%TFA solution using the HTX TM-Sprayer, giving the richest ion populations and ion signal intensities, as well as the clarity of fingermark ridge details. The slides were coated with the parameters in Table 4-4.

Table 4-4. Spray parameters of CHCA matrix onto the latent fingermarks deposited on the glass slides.

Parameters	Optimized values or settings
Concentration of CHCA	5 mg/mL
Flow rate	0.1 mL/min
Spray nozzle velocity	1200 mm/min
Spray nozzle temperature	75°C
Track spacing	3 mm
Number of passes	4
Nitrogen pressure	10 psi
Drying time	2 sec
Nozzle Height	40 mm
Gas flow rate	2 L/min
Pattern	Horizontal/horizontal

4.3.2 Typical MALDI-MS spectra and images of latent fingermarks of different donors

Three donors were asked to deposit sebaceous latent fingermarks, and analysis of the **whole fingermarks** was performed to investigate the presence of endogenous and exogenous chemicals. No fingermark development was performed in this section. Figure 4-9 shows the representative MALDI-MS spectra of three donors in positive mode in *m/z* 100-1000.

Different endogenous species were detected on the three fingermarks deposited by different donors. Table 4-5 shows the tentative identifications of compounds detected by MALDI-MS in latent fingermarks deposited by three different donors. The tentative identifications were made on the basis of *m/z* and the assignments made by previous studies which examined and detected chemicals components in latent fingermarks. For example, oleic acid (with $[M + H]^+$ ion at *m/z* 283.3) was detected in donor #2 only,[160] while cholesterol (with $[M\text{-}H2O + H]^+$ ion at *m/z* 369.4) were successfully detected in donor #3.[167] Various TAGs such as $[TAG(45:1)+Na]^+$ and $[TAG(48:1)+Na]^+$ were also detected at *m/z* 785.9 and 827.3 in donor #3 only.[167] Signal intensities of exogenous chemicals such as DMA ions at *m/z* 304.3 and 332.3 were consistent among the three donors.[155,163,164] As these exogenous contaminate can be found in many antiseptic products, the three donors may have come into contact with these chemical species when they were using the toiletries, alcohol wipes or antiseptic detergents to clean their hands before fingermark deposition. This accidental and unintentional contamination, as well as the detection of the species can prove that MALDI-MS is a very sensitive instrument that can detect both endogenous and exogenous substances simultaneously, even when these species are present in small amounts in each latent fingermark.

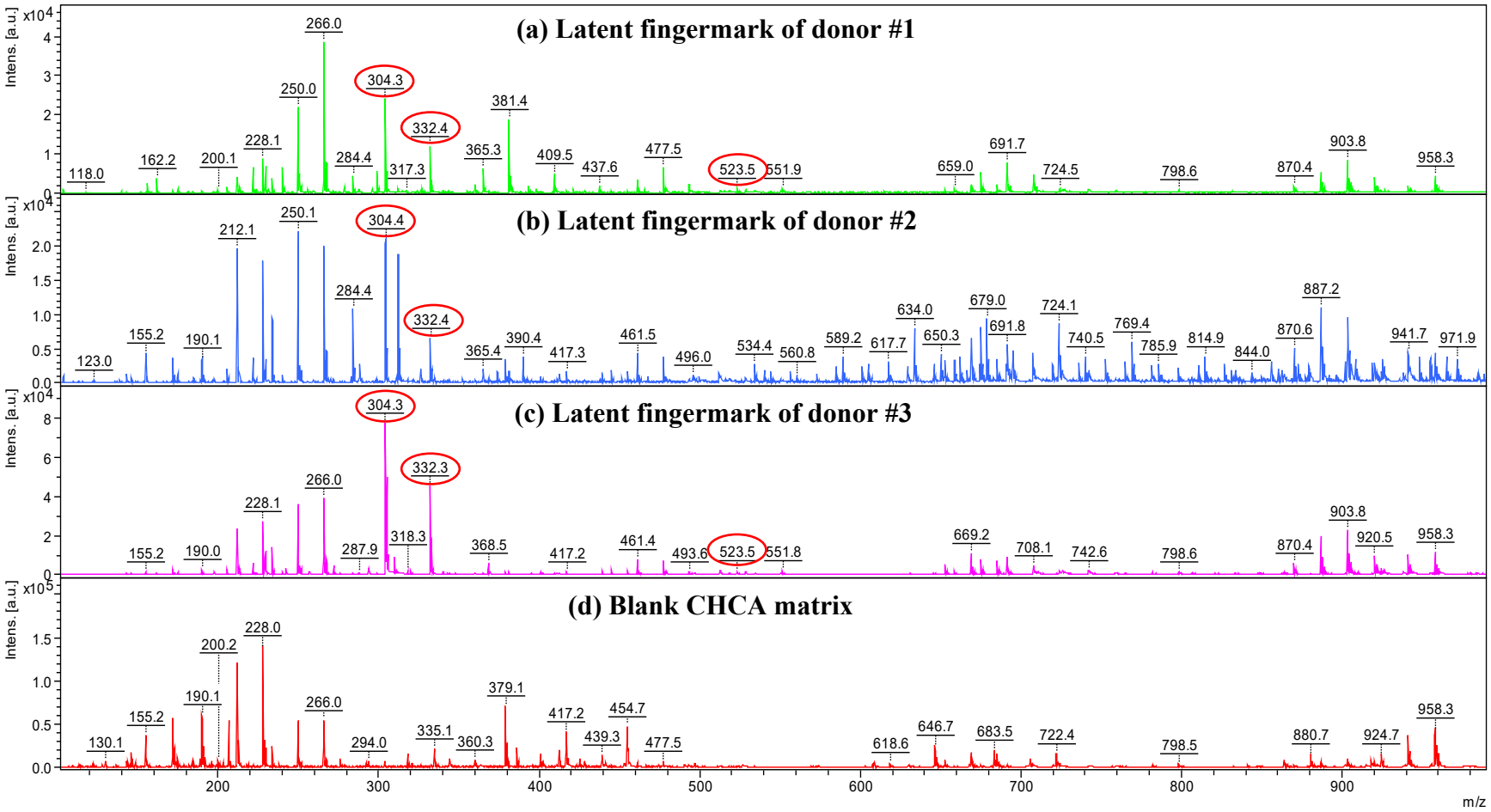


Figure 4-9. MALDI-MS analysis in positive mode between *m/z* 100 and 1000 of three different fingermark donors. (a) Mass spectrum of a latent fingermark of donor #1. (b) Mass spectrum of a latent fingermark of donor #2. (c) Mass spectrum of a latent fingermark of donor #3. (d) Mass spectrum of blank CHCA matrix.

Table 4-5. Compounds detected by MALDI-MS in latent fingermarks deposited by three different donors. All compounds were tentatively identified.

Tentative identification with references		Observed *m/z*	Donor #1	Donor #2	Donor #3
Fatty acids	oleic acid[160]	283.3		✓	
	stearic acid[158-160]	285.3	✓	✓	
	nonadecadienoic acid[160]	295.2			✓
	tetracosadienoic acid[160]	382.4	✓	✓	
Cholesterol	cholesterol[167]	369.4			✓
Squalene	squalene[159,161]	411.2	✓		✓
Exogenous compounds	dimethylbenzylammonium ion, DBA ($12CH_2$)[155,163,164]	304.3	✓	✓	✓
	dimethylbenzylammonium ion, DBA ($14CH_2$)[164]	332.4	✓	✓	✓
	behentrimonium[166]	368.5	✓	✓	✓
	dimethylhexadecyl-octadecylammonium ion[168]	523.5	✓		✓
TAGs	glycerophosphoserine[163]	666.4		✓	
	TAG(45:1)[167]	785.9		✓	
	TAG(48:1)[167]	827.3		✓	

Figure 4-10 shows the MALDI-MS images reconstructed from ion signals of different *m/z*. Fingermark patterns from two different donors were constructed by locating the *m/z* values in order to show the spatial distributions of chemical species. A clear image of the whole fingermark and its ridge pattern was obtained for the distribution of dimethylbenzylammonium ion (*m/z* 304.3).[155,163,164] Similar traces were observed for the cetrimonium ion (*m/z* 284.4).[162] Level 1 details which are important for identification are present on these two MS images, i.e., a whorl pattern for *m/z* 304.3 and an arch pattern for *m/z* 284.4. The identities of the peaks such as *m/z* 184.1 and *m/z* 287.5 could not be identified, however they are not important in MSI analysis as they are only used as particular ions to reconstruct the whole fingermark pattern. Overall, the distributions of all chemical species follow the papillary ridge pattern, and that the matrix ion signals did not interfere with the detection of the chemical species of interest in MSI analysis.

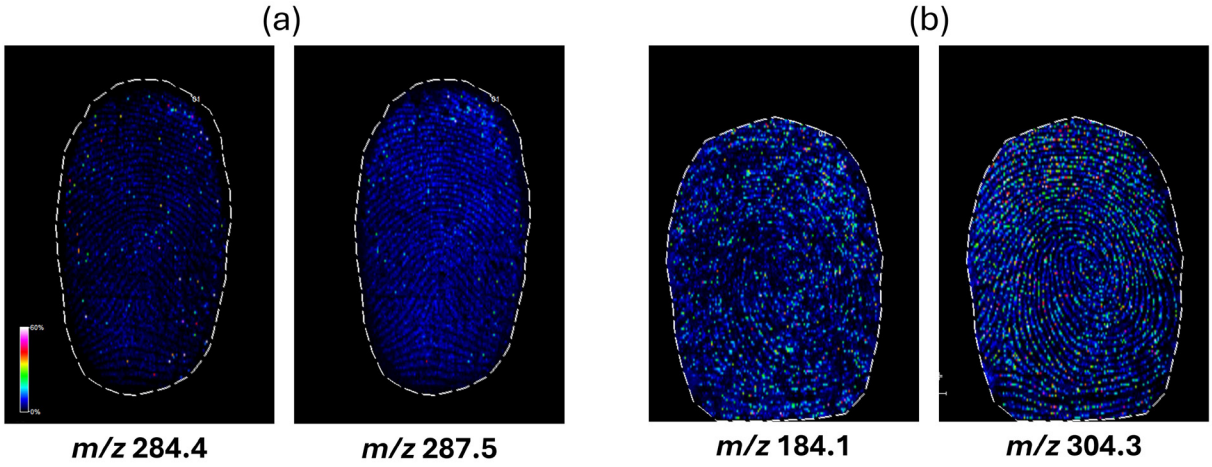


Figure 4-10. MALDI-MS images of latent fingermarks deposited by different donors (a) and (b).

4.3.3 Typical MALDI-MS spectra and images of latent fingermarks sprayed with fire extinguisher dry powders

Comparison of **split fingermarks** was performed for a direct comparison of the parameters from the same fingermark deposition to determine whether the spraying process of fire extinguisher dry powders affects the subsequent MSI for both chemical information and spatial information of endogenous and exogenous chemicals in friction ridges. The spectra and image quality, as well as the intensities of the fingermark compounds were evaluated.

Figure 4-11 and Figure 4-12 show the representative MALDI-MS spectra of latent fingermarks with and without prior development with fire extinguisher dry powders in *m/z* 250-400 and 500-1000 respectively. The signal intensities decreased for both endogenous and exogenous chemicals when the fingermarks were developed with fire extinguisher dry powder. Figure 4-13 and Figure 4-14 show the normalized intensities of examples of endogenous and exogenous compounds in order to investigate the effect of the fire extinguisher dry powder on MALDI-MS profiling. The developed fingermark signal intensity was normalized to the non-developed fingermark signal intensity from the split fingermarks. A normalized intensity of one indicates that there is no signal suppression by fire extinguisher dry powder, proving that the powder is completely compatible with MALDI-MSI. Over half of the endogenous and exogenous compounds were found to have a

normalized intensity of nearly one, proving that fire extinguisher dry powder is compatible with MALDI-MSI in the positive mode.

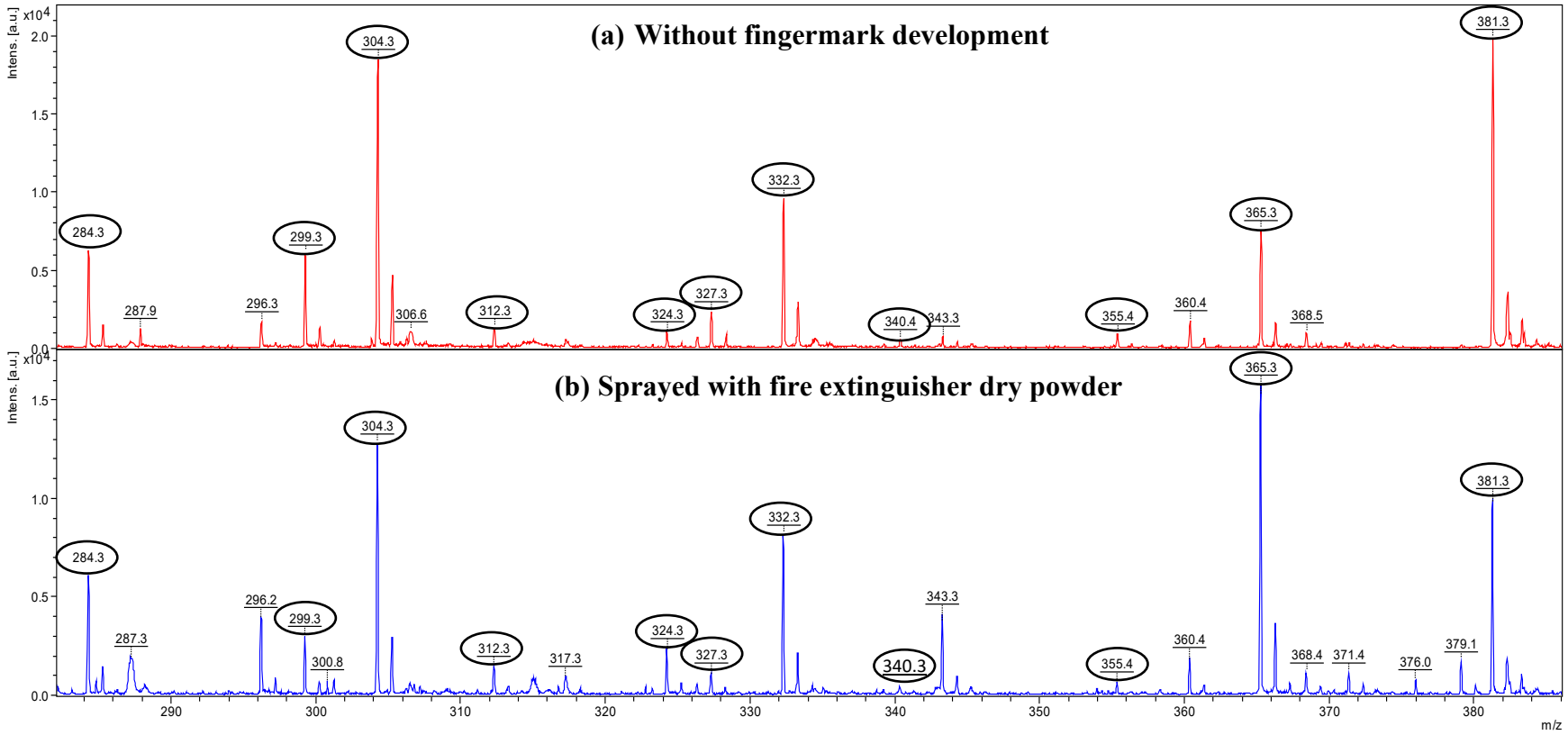


Figure 4-11. Mass spectra obtained in positive mode between *m/z* 250 and 400 in which (a) one half of a fingermark was non-developed and (b) another half of the fingermark was developed with fire extinguisher dry powder.

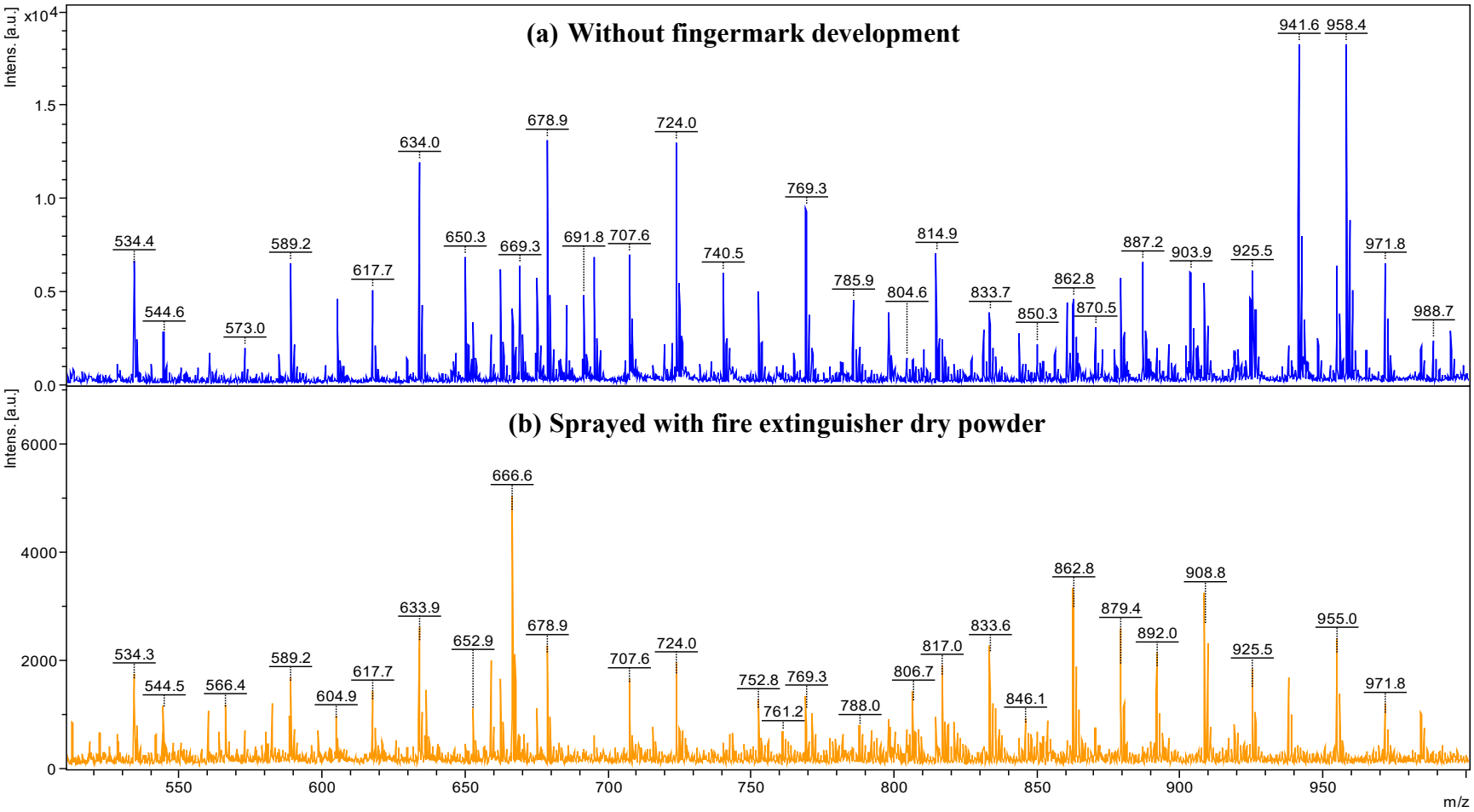


Figure 4-12. Mass spectra obtained in positive mode between *m/z* 500 and 1000 in which (a) one half of a fingermark was non-developed and (b) another half of the fingermark was developed with fire extinguisher dry powder.

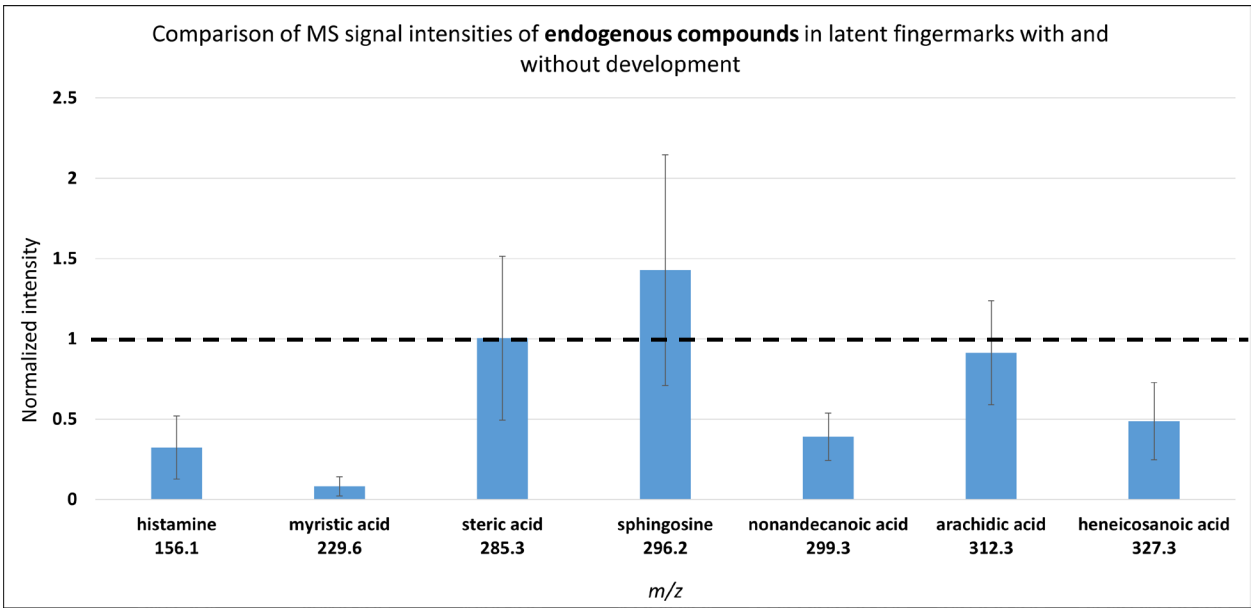


Figure 4-13. Relative intensities of examples of endogenous compounds in developed fingermarks normalized to that of non-developed fingermarks.

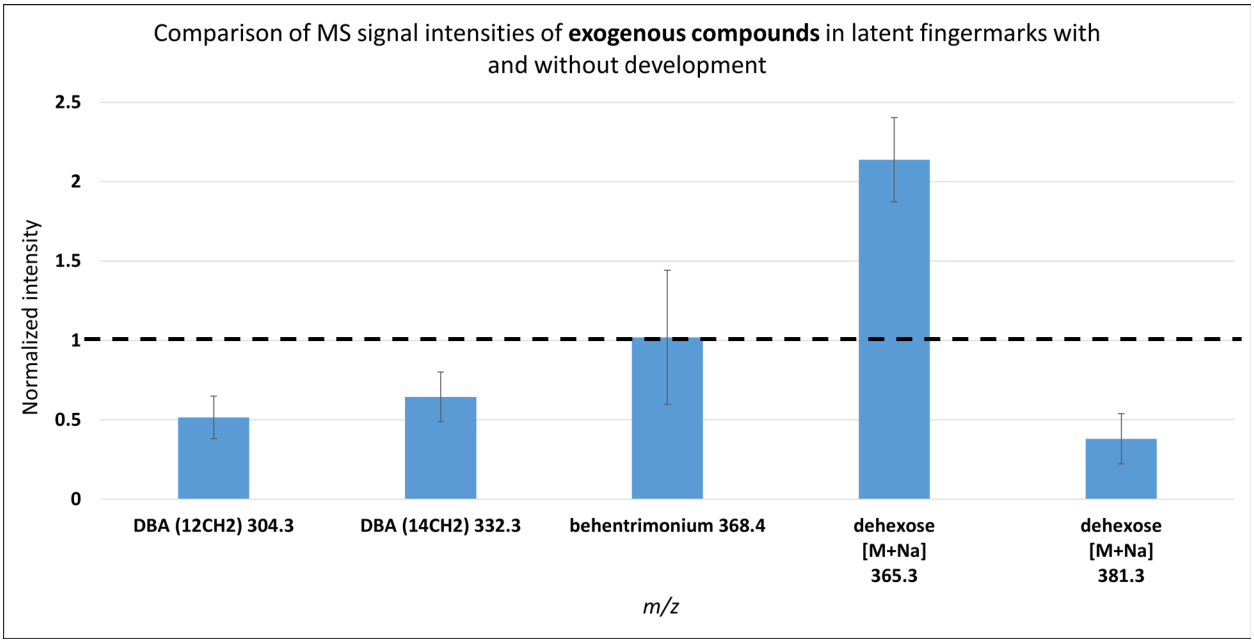


Figure 4-14. Relative intensities of examples of exogenous compounds in developed fingermarks normalized to that of non-developed fingermarks.

Figure 4-15 and Figure 4-16 show the MALDI-MS images reconstructed from ion signals of different *m/z.* Fingermark pattern from the same donor was constructed by locating the *m/z* values in order to show the spatial distributions of chemical species. In Figure 4-15, *m/z* 229.6 for nonandecanoic acid and *m/z* 304.3 for DBA ion were located on the split fingermarks in which the fingermark on the top was non-developed and the fingermark at the bottom was developed with fire extinguisher dry powder. In Figure 4-15, clear images of the whole fingermark and its ridge pattern was obtained for the distribution of TAGs at *m/z* 769.3, *m/z* 891.7 and *m/z* 971.8. Level 1 details which are important for identification can be observed, i.e., an arch pattern. Overall, the distributions of all chemical species followed the papillary ridge pattern and fire extinguisher dry powder did not interfere with the detection of the chemical species of interest in MSI analysis.

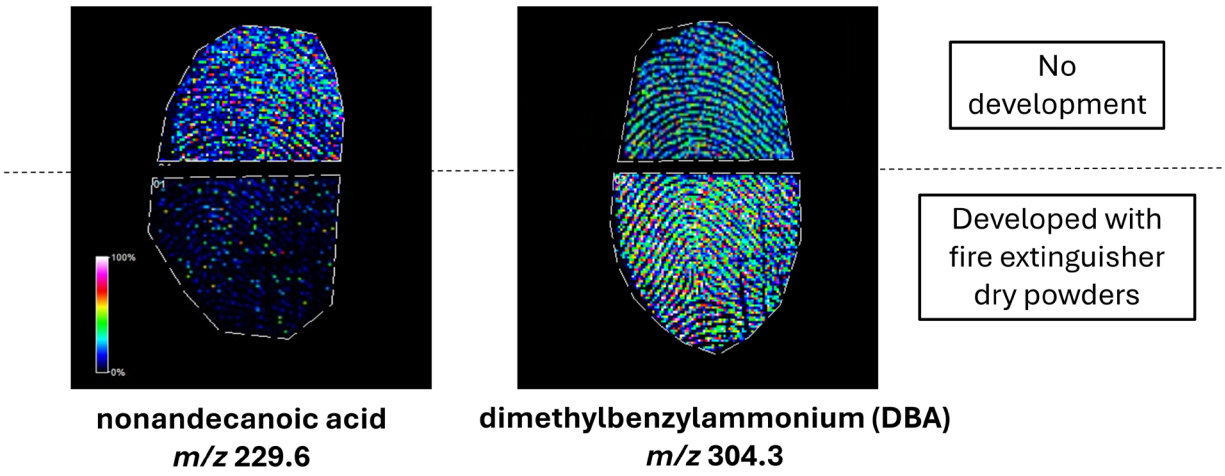


Figure 4-15. MALDI-MS images of split fingermarks at *m/z* 229.6 for nonandecanoic acid and at *m/z* 304.3 for DBA ion, in which the fingermark on the top was non-developed and the fingermark at the bottom was developed with fire extinguisher dry powder.

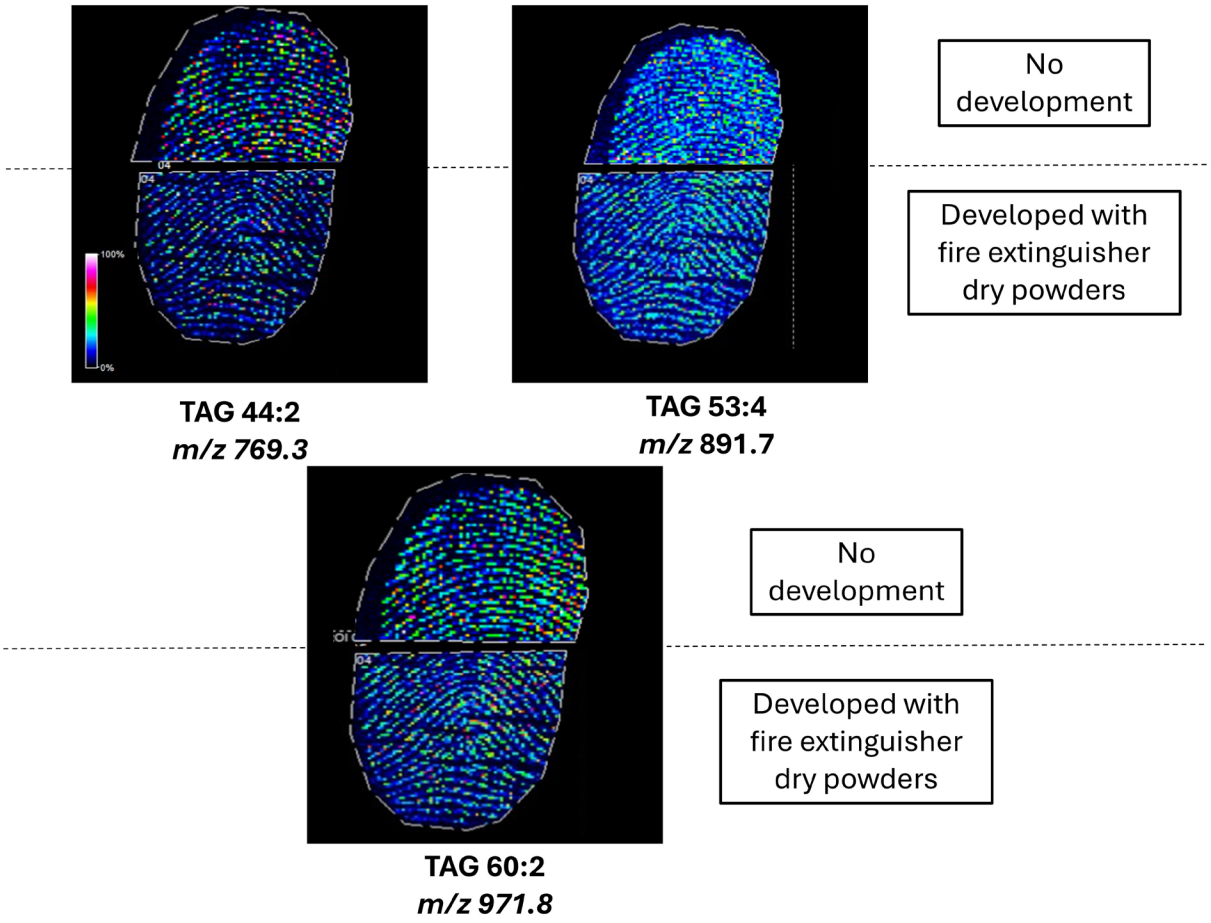


Figure 4-16. MALDI-MS images of split fingermarks at *m/z* 769.3, *m/z* 891.7 and *m/z* 971.8, in which the fingermark on the top was non-developed and the fingermark at the bottom was developed with fire extinguisher dry powder.

After confirming that the spraying process of fire extinguisher dry powders do not affect much the subsequent MSI for profiling of endogenous and exogenous chemicals and imaging of friction ridges, **whole latent fingermarks** were used for analysis instead of split fingermarks and they were sprayed with fire extinguisher dry powders for visualization.

Figure 4-17 and Figure 4-18 show the representative MALDI-MS spectra of a whole latent fingermark with and without prior development with fire extinguisher dry powders. The signal intensities decreased slightly for both endogenous and exogenous chemicals when the fingermark was developed with fire extinguisher dry powder.

Figure 4-19 shows the MALDI MS images reconstructed from ion signals of different *m/z.* The MS images showing the spatial distribution of various endogenous and exogenous substances had ridge pattern identical to the optical image of the developed fingermark which could be used for comparison purposes. Level 1 details which are important for identification are present on all MS images, i.e., a whorl pattern. Overall, the distributions of all chemical species followed the papillary ridge pattern, and that fire extinguisher dry powder did not interfere with the detection of the chemical species of interest in MSI analysis, giving excellent ridge details.

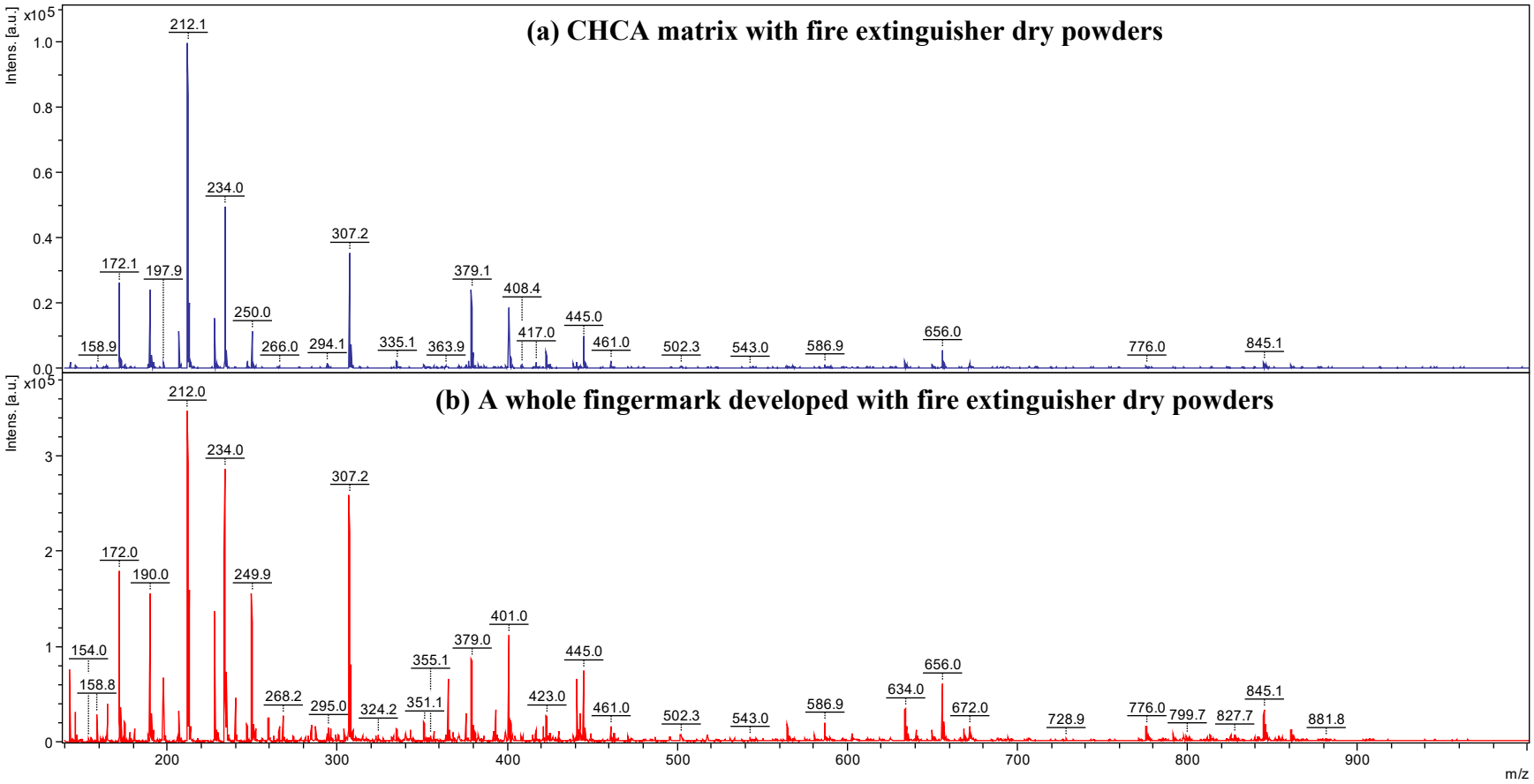


Figure 4-17. MALDI-MS analysis in positive mode. (a) Mass spectrum of CHCA matrix and fire extinguisher dry powders. (b) Mass spectrum of a whole fingermark sprayed with fire extinguisher dry powders and CHCA matrix.

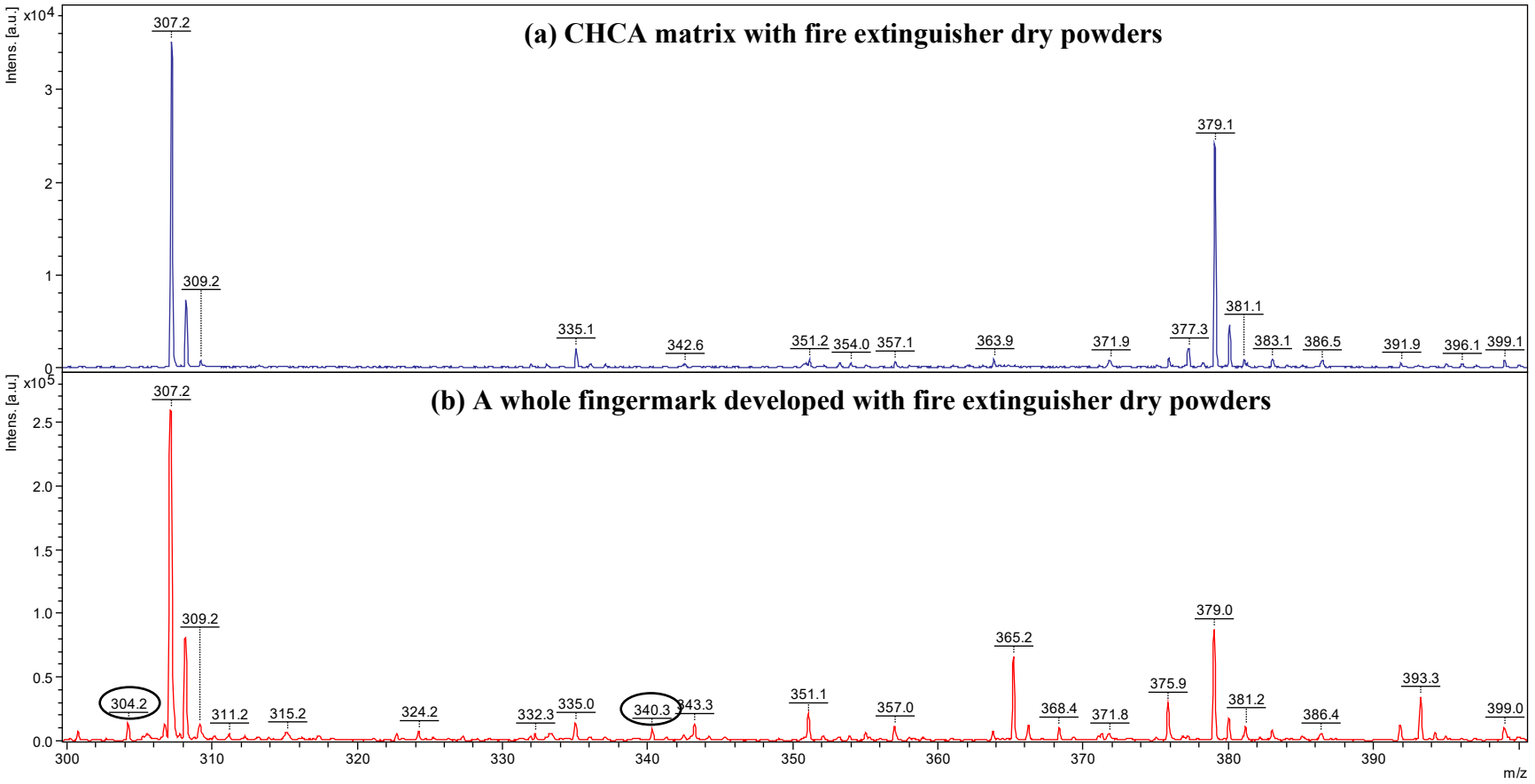


Figure 4-18. MALDI-MS analysis in positive mode between *m/z* 300 and 400. (a) Mass spectrum of CHCA matrix and fire extinguisher dry powders. (b) Mass spectrum of a whole fingermark sprayed with fire extinguisher dry powders and CHCA matrix.

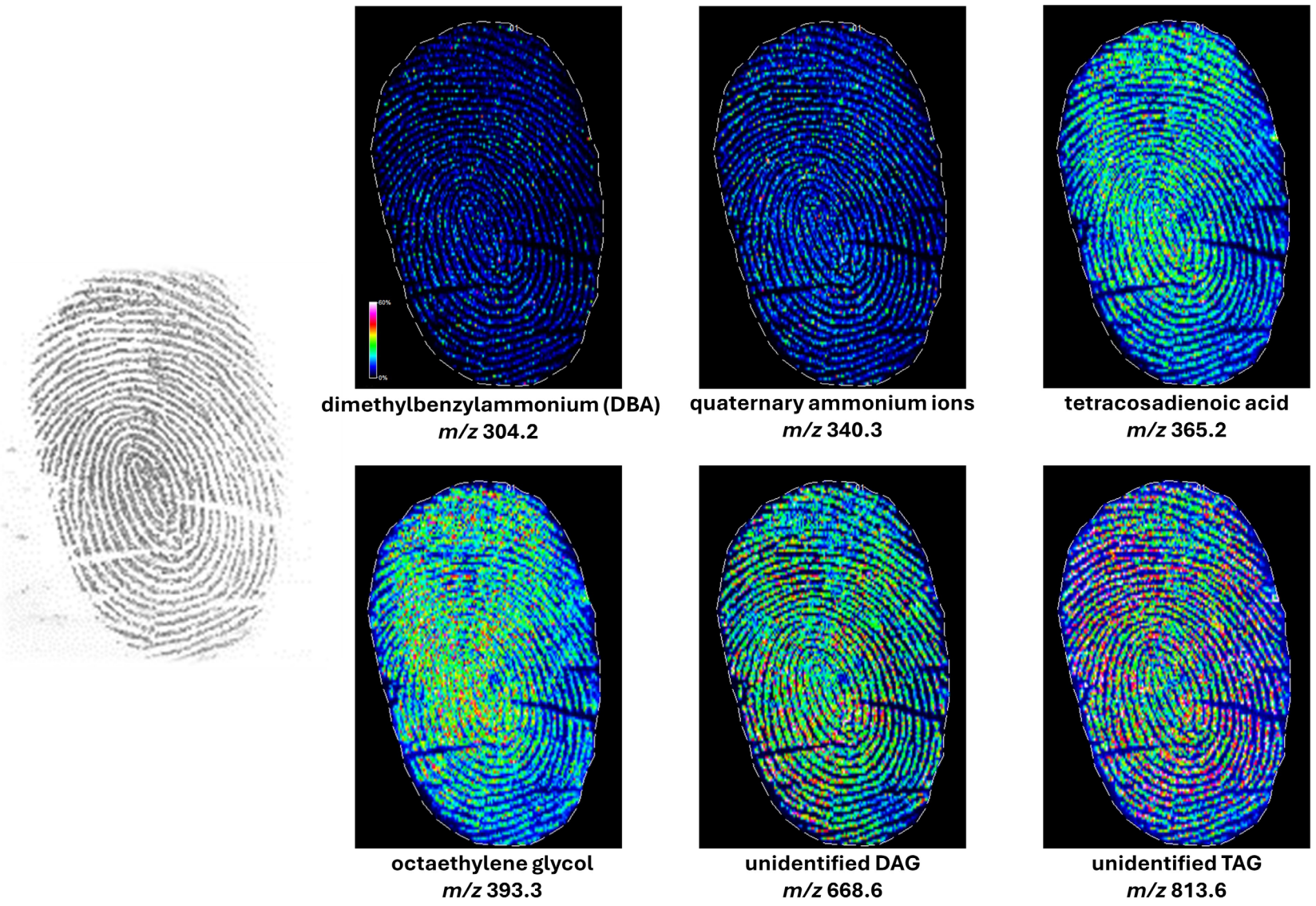


Figure 4-19. MALDI MS images of a whole fingermark developed with fire extinguisher dry powders at different *m/z* and the optical image of the fingermark pattern for comparison purposes.

Exogenous compounds are present due to external contamination. By finding out the identities of some commonly available contaminants, an individual's lifestyle and recent activities can be reconstructed.[148-149] Three case scenarios are now provided where the value of fingerprints was enhanced using chemical imaging after developing with fire extinguisher dry powders.

1) Commonly used personal and household products

On the fingermarks deposited by the three donors, some external contaminants (*m/z* 550.6, 522.6, and 494.6) which were reported to be quaternary ammonium species that contain long-chain hydrocarbon groups were identified before and after the development of fire extinguisher dry powders.[168] Figure 4-20 shows the mass spectra of latent fingermarks deposited by the three donors after the fingermarks were developed with fire extinguisher dry powders. However, the signal intensities of these three external contaminants vary among the three donors, with one donor reaching 1 x 10^6 level in signal intensities. Contaminant at *m/z* 550.6 is a dimethyldioctadecylammonium (DDA) ion and commonly appears in mass spectra along with *m/z* 522.6 and 494.6 which belong to the same family as ditallowdimethylammonium ions but have shorter alkyl-chain groups.[168] These compounds are lipophilic in nature, with surfactant and antistatic softening properties.[169-170] They are reported to be widely available in personal and

household products such as body lotions, hair products, detergents, wipes, fabric softeners, body washes and paper used for tissue products.[168-170]

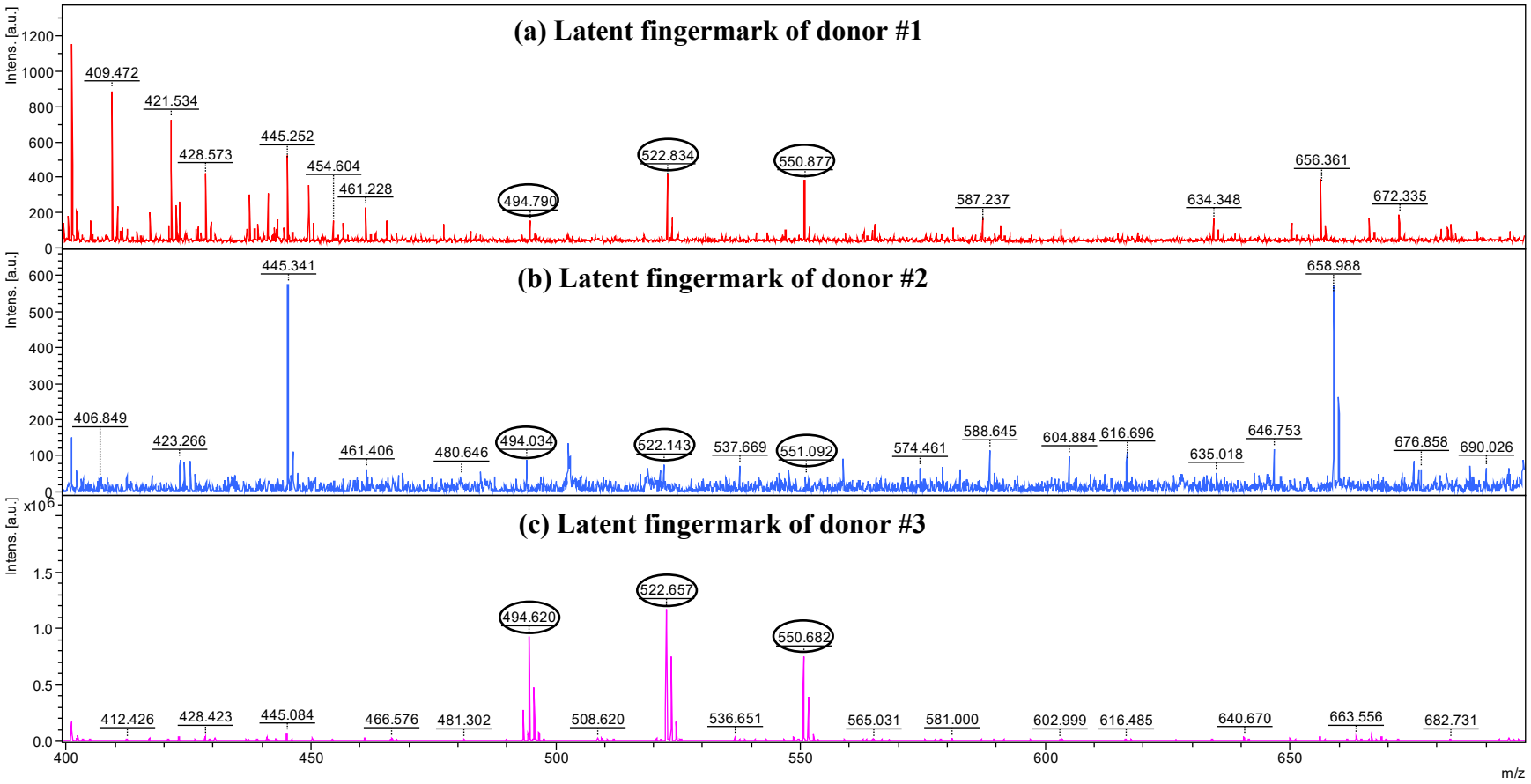


Figure 4-20. MALDI-MS analysis in positive mode between *m/z* 400 and 1000, showing the tentative identifications of exogenous contaminants at *m/z* 550.6, 522.6 and 494.6 in fingermarks deposited by three donors after spraying with fire extinguisher dry powder. (a) Mass spectrum of a latent fingermark of donor #1. (b) Mass spectrum of a latent fingermark of donor #2. (c) Mass spectrum of a latent fingermark of donor #3.

Figure 4-21 shows the normalized intensities of these three external contaminants in order to investigate the effect of the fire extinguisher dry powder. The developed fingermark signal intensity was normalized to the non-developed fingermark signal intensity from the split fingermarks. A normalized intensity of one indicates that there is no signal suppression by the development procedure by fire extinguisher dry powder, proving that the powder is completely compatible with MALDI-MSI. These three external contaminants were found to have normalized intensities of nearly one, proving that fire extinguisher dry powder is compatible with MALDI-MSI in the positive mode.

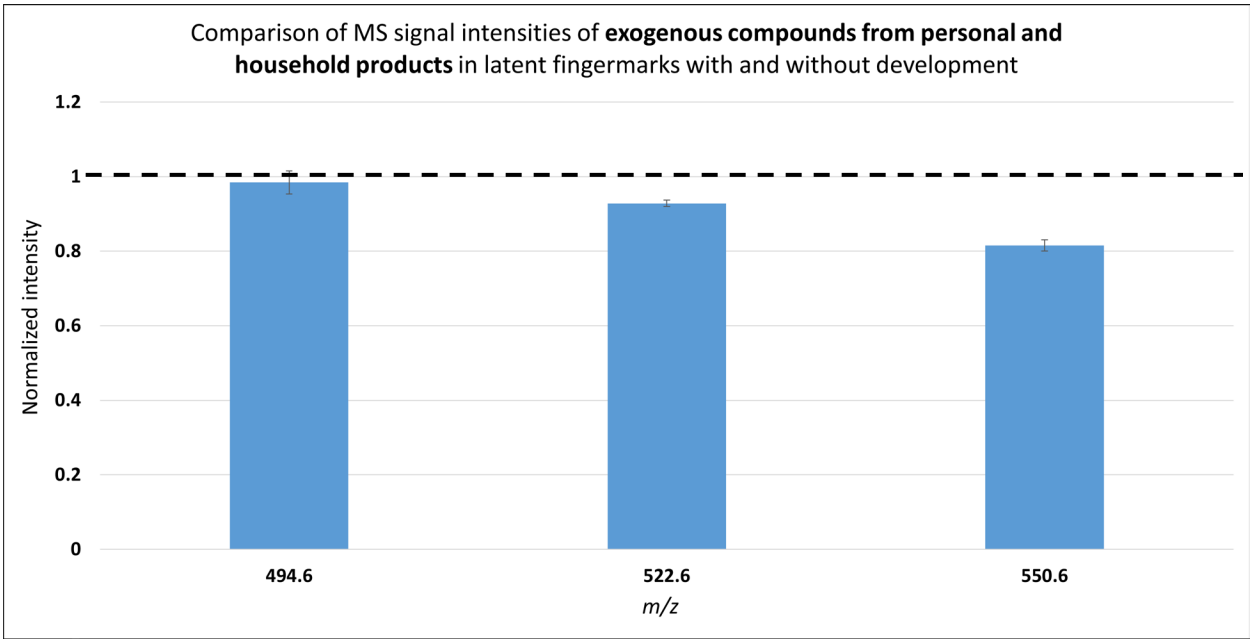


Figure 4-21. Relative intensities of the three exogenous contaminants from commonly used personal and household products in developed fingermarks normalized to that of non-developed fingermarks.

Figure 4-22 shows the MALDI-MS images at *m/z* 550.6 of fingermarks deposited by two different donors. MALDI-MSI analysis enabled the differentiation of fingermarks deposited since the DDA ion at *m/z* 550.6 was found to be absent from one donor.

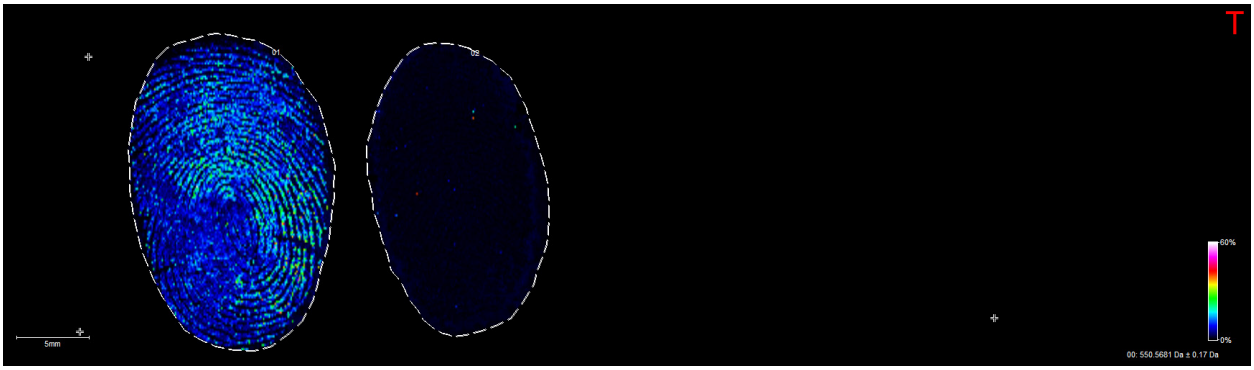


Figure 4-22. MALDI-MS images of two fingermarks deposited by two different donors, showing the distributions of DDA ion at *m/z* 550.6.

Figure 4-23 shows the representative MALDI-MS spectra of different positions of the same fingermark deposited from the same donor, after spraying with fire extinguisher dry powders. Exogenous contaminants at *m/z* 550.6, 522.6 and 494.6 were identified with similar intensities of the peaks reaching 1 x 10^6. Overall, the characteristic spectral patterns of the same latent fingermark developed with fire extinguisher dry powder were highly reproducible regardless of the position of the fingermark for MALDI-MS profiling and imaging.

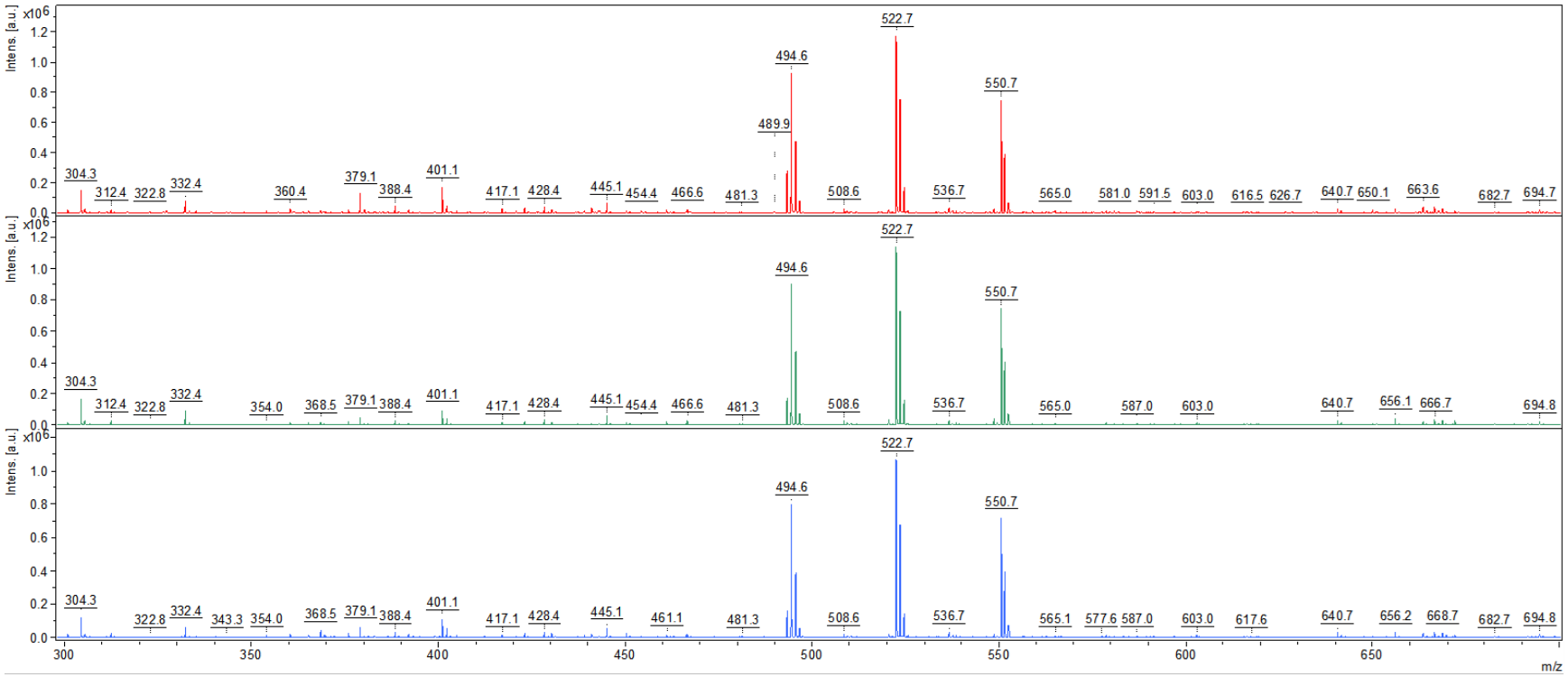


Figure 4-23. Representative MALDI-MS spectra showing the tentative identifications of exogenous contaminants at *m/z* 550.6, 522.6 and 494.6 at different positions of the same fingermark deposited from the same donor, after spraying with fire extinguisher dry powders.

It is always important to obtain MS/MS spectra and compare with the MS/MS spectra from standards. Unambiguous identification via MS/MS fragmentation can prove the chemicals present in the latent fingermarks which can then be admissible in the court of law. The MS/MS spectra of the three external contaminants (*m/z* 550.6, 522.6, and 494.6) in Figure 4-24 are also in agreement with those reported in previous studies.[168]

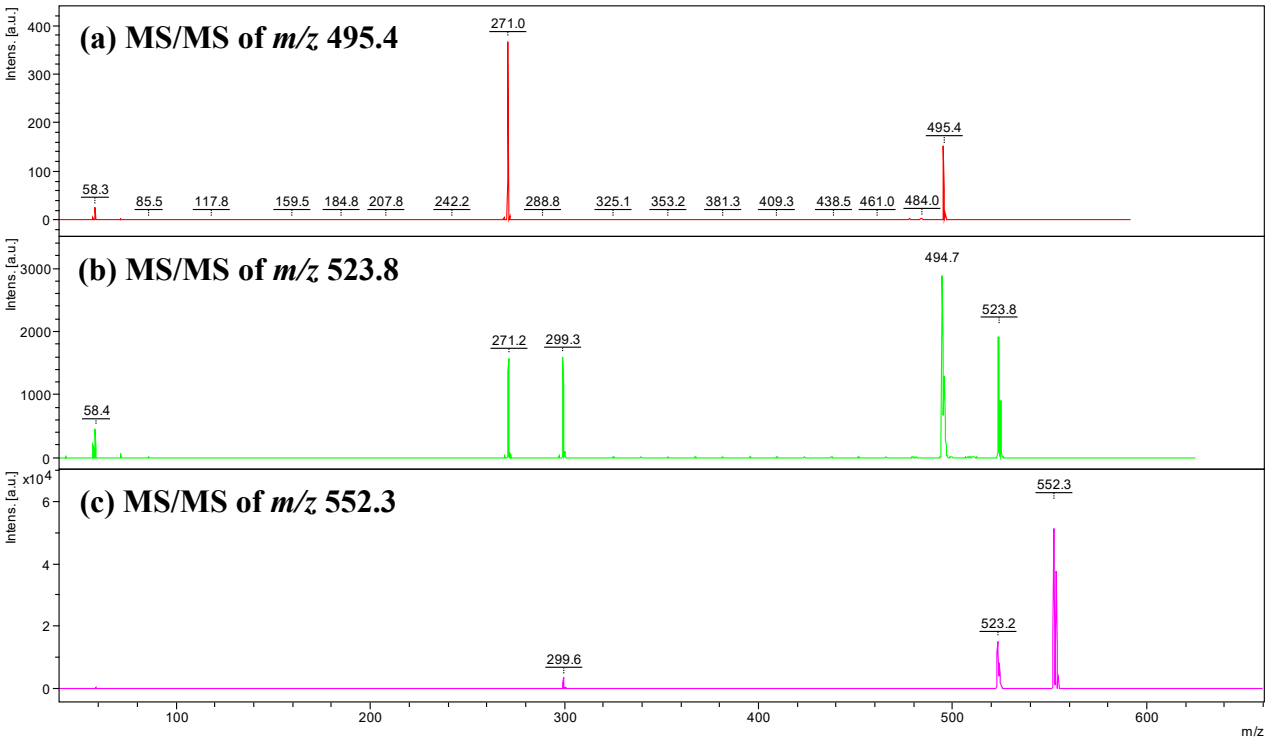


Figure 4-24. MS/MS spectra of exogenous contaminants at (a) *m/z* 495.4, (b) *m/z* 523.8 and (c) *m/z* 552.3.

Taking the DDA ion (C_{18}, C_{18}) at *m/z* 552.3 as an example in Figure 4-25, MS/MS fragmentation of the ion gives one dominant ion at *m/z* 299 with some smaller mass hydrocarbon ions at 57 and 81. This is because the structure has two identical C_{18} side chains, which gives a fragment ion of $C_{20}H_{44}N$ at *m/z* 299 under MS/MS.[168] The MS/MS spectrum of *m/z* 523 gives ions at *m/z* 271 and 299, therefore the precursor ion is likely to be dimethylhexadecyloctadecylammonium (C_{16}, C_{18}).[168] The MS/MS spectrum of *m/z* 495 gives one dominant ion at *m/z* 271, therefore the precursor ion is a dimethyldihexadecylammonium ion (C_{16}, C_{16}).[168]

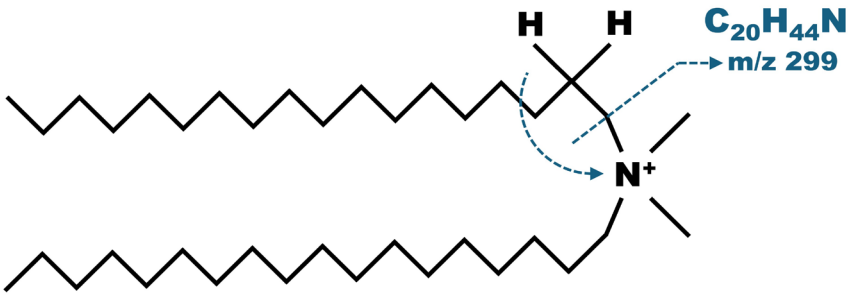


Figure 4-25. Chemical structure of dimethyldioctadecylammonium ion at *m/z* 552.3 and its fragmentation pattern.[168]

2) Condom

Two widely available brands of condom namely *Trojan* and *Okamoto* which use a water-soluble gel-type lubricant with nonoxynol-9 (C_9H_{19}-C_6H_4-$(OCH_2CH_2)_n$OH where n is 9) as a spermicide in its formulation were investigated. Fingerprint donors were asked to handle the condom with sebum-rich fingers, mimicking a real usage prior to fingermark deposition, and subsequently, MALDI-MS was used to identify traces of nonoxynol-9 from latent fingerprints with and without prior development with fire extinguisher dry powders. Figure 4-26 shows the mass spectra of two condom-contaminated fingermarks after the fingermarks were developed with fire extinguisher dry powders. For both condom brands, the MALDI MS spectra showed the presence of three ion series. Taking *Okamato* as an example, the three ion series were (i) sodiated nonoxynol-9 with ions from *m/z* 463.1 to 1431.6 with a formula of 203 + n(44) + 17 + 23 and a Gaussian maxima centered at *m/z* 727.2 (11-ethoxymer); (ii) potassiated nonoxynol-9 with ions from *m/z* 523.1 to 1315 with a formula of 203 + n(44) + 17 + 39 and a Gaussian maxima centered at *m/z* 787.2; (iii) octoxynol-9 (C_8H_{17}-C_6H_4-$(OCH_2CH_2)_n$OH where n is 9) which is another ionic surfactant and spermicide, with ions from *m/z* 713.2 to 887.3 with a formula of 189 + n(44) + 17 + 23 and a Gaussian maxima centered at *m/z* 844.9. The ion signals of the polymer series were spaced by a mass difference of 44 amu. This was due to the presence of an ethoxylate monomer as repetition unit. The detection of both the spermicides nonoxynol-9 and octoxynol-9 in *Okamato* has not yet been reported, while the detection of both species in *Trojan* agreed with the results reported by

others.[164] Although nonoxynol-9 and octoxynol-9 were detected in both brands (while only nonoxynol-9 is stated in the formulation), it is clear that the detection of the chemicals alone could not be used to achieve condom brand identification and differentiation, which requires further investigation. This case scenario is important at a crime scene of sexual assault, as handling of a condom would transfer the coating of the condom onto the perpetrator's fingerprints.

Figure 4-27 shows the MALDI-MS images of a potassiated nonoxynol-9 ion at *m/z* 875.6 and a sodiated nonoxynol-9 at *m/z* 1211.8 in four fingermarks which were developed with fire extinguisher dry powder. As the middle two fingermarks were deposited without prior contact with a condom, these ions were not present, allowing the four deposited fingermarks to be differentiated. In the current stage, it is proved that the development of the latent fingermarks with fire extinguisher dry powder did not interfere with the detection of the chemical species of interest (i.e., spermicide in condom lubricants) in MALDI-MSI profiling and imaging.

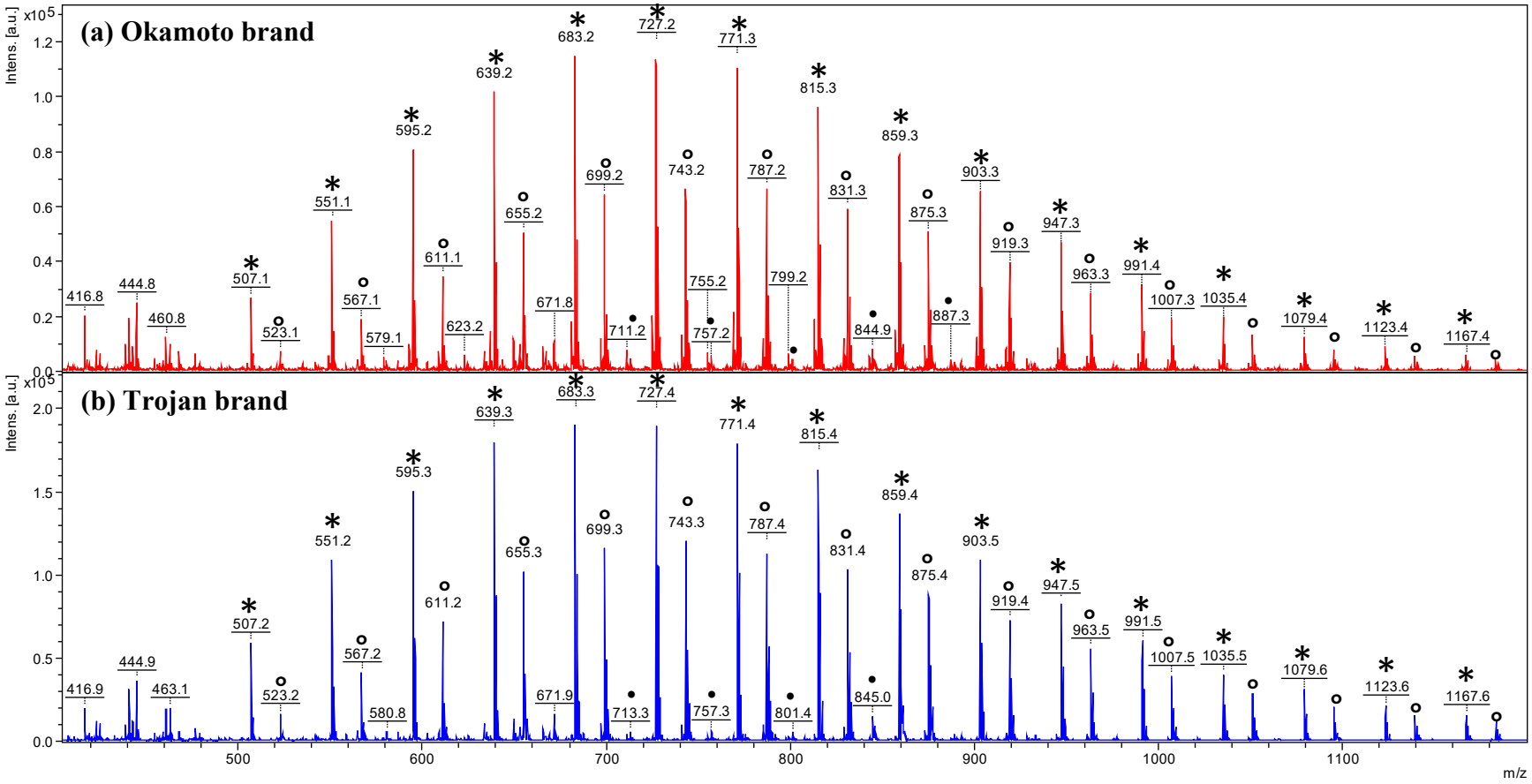


Figure 4-26. Representative MALDI-MS spectra obtained for fingerprints in which the donor came into contact with 2 brands of condom lubricant before fingerprint deposition with sodiated nonoxynol-9 ion series as *, potassiated nonoxynol-9 ion series as ° and octoxynol-9 ion series as •. The two condom brands are (a) Okamoto and (b) Trojan.

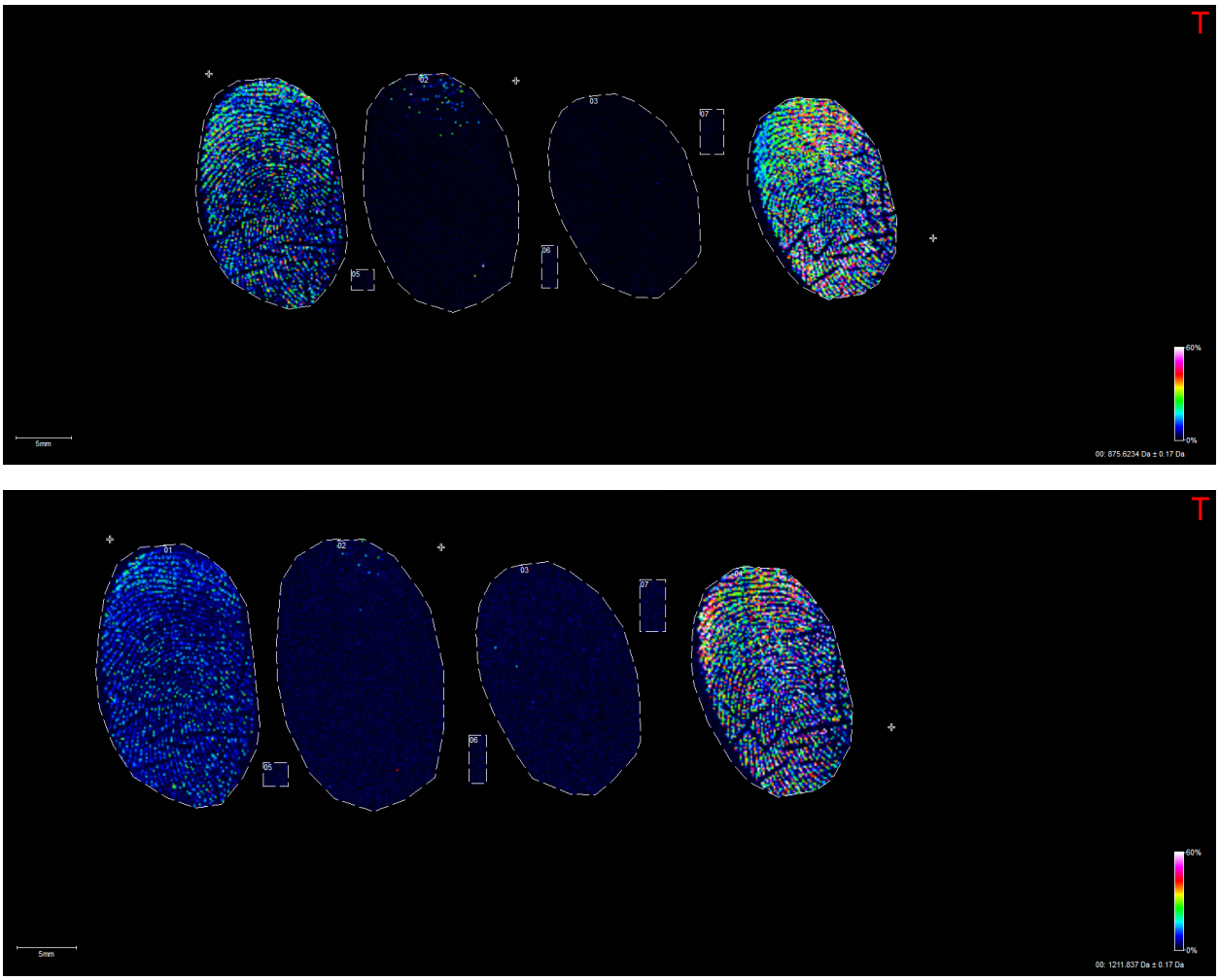


Figure 4-27. MALDI-MS images of four fingermarks in which the middle two fingermarks were deposited without prior contact with a condom, showing the absence of ions at *m/z* 875.6 and 1211.8 which are in the potassiated nonoxynol-9 ion series.

3) Over-the-counter drug - aspirin

Figure 4-28 describes the relative abundance of the aspirin tablet constituent, acetylsalicylic acid, on a latent fingermark developed with fire extinguisher dry powder. The spatial distributions were revealed by extracting the relative abundance of the ions $[M+Na]^+$ at *m/z* 203.1, while the remaining fingermark ridge pattern could be shown by extracting an unidentified ion at *m/z* 272.2. The distribution of acetylsalicylic acid resembled not only the shape of the tablet but also its surface and grooves, giving some hints on the brand of the drug. This experiment demonstrated how fire extinguisher dry powder and chemical imaging can be employed together effectively in establishing a direct link between a person and the materials the person has encountered previously.

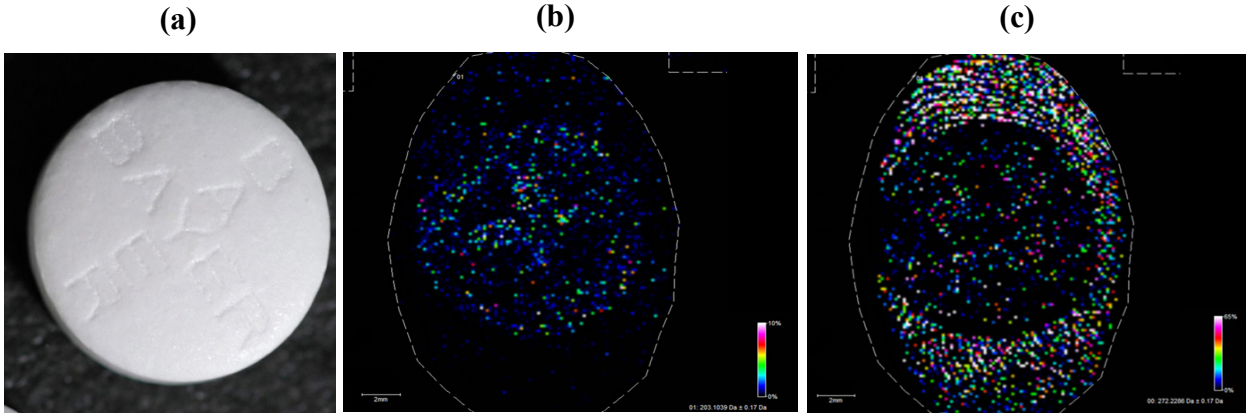


Figure 4-28. Detection of exogenous compounds in a latent fingermark sprayed with fire extinguisher dry powder. (a) Optical image of a round aspirin pill which the fingermark donor came into contact with; (b) MALDI-MS image of acetylsalicylic acid $[M+Na]^+$ (*m/z* 203) and (c) MALDI-MS image of *m/z* 272, corresponding to an unidentified ion.

4.3.4 MALDI-MSI separation of overlapping fingermarks sprayed with fire extinguisher dry powders

Overlapping fingermarks are commonly encountered in crime scenes, however they present a great challenge to forensic scientists due to the difficulties in distinguishing two or more ridge patterns and finding out the donors (e.g., the victim or the offender) who have generated the fingermarks.

In the preparation of the overlapping latent fingermarks (condom-contaminated fingermark and groomed fingermark) containing different chemical compounds, a donor was asked to handle a condom as if being consumed prior to deposition of the vertical fingermark, while another donor was asked to deposit a sebaceous fingermark horizontally on the top, overlapping a small part with the vertical fingermark. Figure 4-29 shows the MALDI-MSI separation of overlapping fingermarks after development with fire extinguisher dry powders. Discernible friction ridges were clearly observed after development and the matrix application did not visually distort the fingermarks. MALDI-MSI separation of the two fingermarks was demonstrated through the selection of unique ion signals of each fingermark. The vertical fingermark was constructed by locating a potassiated nonoxynol-9 ion at *m/z* 831.8, and the image showed good ridge pattern clarity even in the area that was originally overlapped. Due to the presence of a potassiated nonoxynol-9 ion at *m/z* 831.8 and ion series in the mass spectrum, the fingermark donor's recent

activities could be potentially reconstructed, i.e., prior contact with a condom before fingermark deposition. The horizontal fingermark was constructed by locating the behentrimonium ion at *m/z* 368.5, which was reported to be one of the exogenous quaternary ammonium ions found in lotions.[166] Again, the image showed good ridge pattern clarity with level 1 details being observed i.e., a loop pattern type. Based on the presence of some specific compounds in the respective molecular images of the overlapping latent fingermarks, the two latent fingermarks could be separated in one single analysis, proving that the development of latent fingermarks with fire extinguisher dry powder can allow the unambiguous separation and differentiation of the overlapping fingermarks, which are common to encounter in crime scenes.

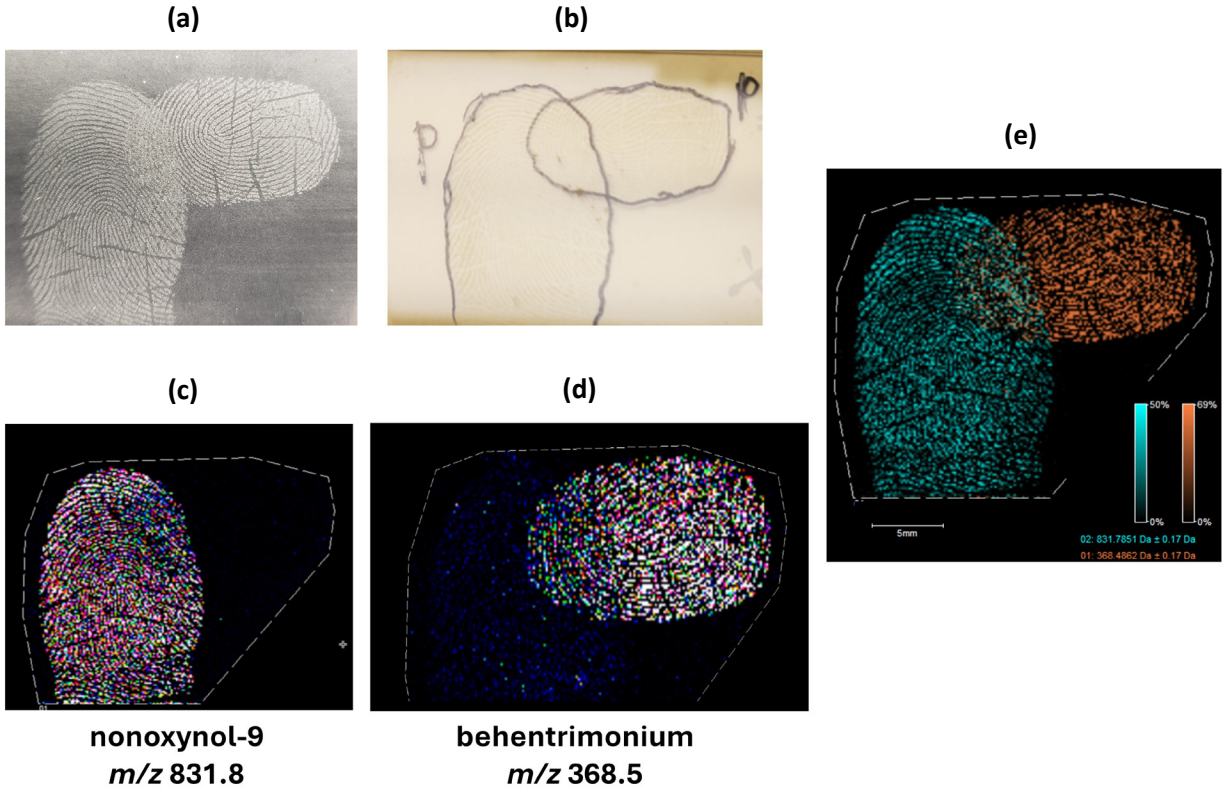


Figure 4-29. MALDI-MSI separation of overlapping fingermarks developed with fire extinguisher dry powder. (a) Optical image of the overlapping fingermarks deposited by two different donors (donors 1 and 2) and developed with fire extinguisher dry powders. (b) Optical image of the overlapping fingermarks after matrix spraying. (c) Image of the potassiated nonoxynol-9 ion at *m/z* 831.8. (d) Image of the behentrimonium ion at *m/z* 368.5. (e) Image of the two overlapping fingermarks by their respective molecular images.

4.4 Conclusions

A new and novel fingermark detection method - fire extinguisher dry powder was demonstrated to be quick, simple, cost-effective, sensitive and selective to the moisture and oily components in the fingermark residues, thus offering clear and sharp images of fingermark ridges. To be incorporated into the field of forensic science, the use of fire extinguisher dry powder must be compatible with MALDI-MSI which is a powerful analytical tool to provide both chemical information and spatial information of endogenous and exogenous chemicals in friction ridges in one single analysis.

MALDI-MS protocol for fingermark analysis was first optimized. Three parameters such as matrix selection, matrix concentration and number of passes of matrix were tested to establish a protocol that can produce high quality and reproducible spectra. The best results were achieved in the MS positive mode when using 4 passes of CHCA at a concentration of 5 mg/mL in 70:30ACN/ 0.1%TFA solution using the HTX TM-Sprayer, giving the richest ion populations and ion signal intensities, as well as the clarity of fingermark ridge details.

The spraying process of fire extinguisher dry powder to develop the latent fingermarks allows the direct analysis of endogenous compounds (e.g., amino acids, fatty acids, cholesterol esters, DAGs, WEs and TAGs) and exogenous compounds (due to prior handling of a condom, an aspirin pill or personal and household products) embedded in the fingermarks, as well as the imaging of their distributions without disturbing the fingermark patterns. The simultaneous visualization of latent fingermarks and the recording of its spatial images not only provide valuable evidence about the individual such as his/her lifestyle and recent activities, but also resolve overlapping fingermarks. The feasibility of using MALDI-MSI with fire extinguisher dry powder as the new *in-situ* fingermark development technique was demonstrated, showing the significant potential to be integrated into the routine fingermark forensic analysis. This type of work has never been reported and could be highly useful to help forensic scientists utilizing the full potential of latent fingermarks.

Chapter 5: Overall conclusion and prospects

MS was used to investigate issues in environmental science and forensic science to understand and address the environmental challenges to protect the environment, as well as to create new and novel protocols for the collection and analysis of latent fingermarks at crime scenes with the needs of criminal investigations.

In Yuen Long Creek, due to the high concentrations being detected (in the range of μg/L) and a high R value of over 25, chloroxylenol is found to be a potential stressor which possess a high ecological risk to aquatic organisms. As chloroxylenol is not included in the routine river water quality monitoring programme implemented by the Government, monitoring programs with continual sampling should be taken place. Furthermore, the removal efficiencies of chloroxylenol were found to be strongly dependent on the technology implemented in the STPs. As only 10.4 to 27.9% of chloroxylenol was removed in STPs with chemically enhanced primary treatment (CEPT) such as screening and sedimentation as the wastewater treatment technology, biological treatment should be taken place as it was found to be highly effective in removing chloroxylenol due to microbial degradation. The results of this study could help health and environmental authorities to plan policies, as well as to raise public awareness of the potential ecological consequences of the use of products containing chloroxylenol.

Fire extinguisher dry powder was demonstrated to be quick, simple, cost-effective, sensitive and selective to the moisture and oily components in the fingermark residues, thus offering clear and sharp images of fingermark ridges. It was also found to be compatible with MALDI-MSI as the simultaneous visualization of latent fingermarks and the recording of its molecular images not only provide valuable evidence about the individual such as his/her lifestyle and recent activities, but also resolve overlapping fingermarks. The feasibility of using MALDI-MSI with fire extinguisher dry powders as the new *in-situ* fingermark development technique was demonstrated, showing the significant potential to be integrated into the routine fingermark forensic analysis.

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