FORENSIC PROFILING OF METHAMPHETAMINE TABLETS BY ATTENUATED TOTAL REFLECTANCE-FOURIER TRANSFORM INFRARED SPECTROSCOPY

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by

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Certificate

This is to certify that the dissertation called entitled Forensic profiling of methamphetamine tablets by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy is Bonafide record of research work done by Muhammad Afiq Haikal Fairus Niza during the period from October 2024 – January 2025 under my supervision. I have read this dissertation and that in my opinion it confirms acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfillment for degree of Bachelor of Science in Forensic Science with Honours.

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Declaration

I therefore declare that this dissertation is the result of my own research, unless otherwise mentioned and officially acknowledged. I further declare that it has not previously or concurrently been submitted in its entirety for any other degrees at Universiti Sains Malaysia or any other institution. I allow Universiti Sains Malaysia permission to utilize the dissertation for teaching, research, and promotion.

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(Muhammad Afiq Haikal Fairus Niza)

Date: 27.2.2025

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
ATR	Attenuated total-reflectance
ATS	Amphetamine type stimulant
et al.	*Et alia- and others*
FTIR	Fourier transform infrared
FMA	N-formylmethamphetamine
HPLC	High performance liquid chromatography
PLSR	Partial least squares regression
GC-MS	Gas chromatography-mass spectroscopy
IR	Infrared
PC	Principal component
PCA	Principal component analysis
UNODC	United Nations Drug Control program
UV-vis	Ultraviolet visible spectroscopy

FORENSIK PROFIL UNTUK METHAMPHETAMINE DENGAN PENGGUNAAN SPEKTROSKOPI INFRA MERAH JELMAAN FOURIER-PANTULAN KESELURUHAN DIKECILKAN (ATR-FTIR)

ABSTRAK

Profiling dadah terlarang memainkan peranan penting dalam sains forensik, membantu pihak berkuasa untuk mengesan rangkaian pengedaran dadah, mengenal pasti trend bahan adukan dan memperkukuh penyiasatan berkaitan dadah. Kajian ini menerokai penggunaan spektroskopi infra merah jelmaan Fourier-pantulan keseluruhan dikecilkan (ATR-FTIR) yang digabungkan dengan analisis komponen utama (PCA) untuk memprofilkan tablet methamphetamine yang dirampas di Malaysia. Dalam kajian ini, sebanyak 161 sampel tablet methamphetamine yang dirampas dari negeri Kelantan telah dianalisis menggunakan spektroskopi ATR-FTIR. Seterusnya, profil ATR-FTIR bagi sampel tersebut telah dikenakan dengan PCA untuk pengumpulan dan pembezaan. Hasil kajian menunjukkan bahawa kebanyakan sampel mengandungi tahap bahan adukan yang tinggi, dengan kafein yang paling dominan. Kajian ini juga mendedahkan bahawa sebahagian besar sampel yang mempunyai 157 sampel dan mempunyai 97.5 peratus mengandungi kurang daripada 25% methamphetamine, mencadangkan amalan pengadukan yang meluas dengan tujuan meningkatkan margin keuntungan sambil mengekalkan kesan perangsang.Kesimpulannya, kajian ini membuktikan bahawa spektroskopi ATR-FTIR yang digabungkan dengan PCA menawarkan kaedah analisis dadah forensik yang pantas, tidak merosakkan sampel, dan kos efektif untuk pemprofilan forensik sampel dadah. Hal ini membolehkan penganalisis forensik mengesan sumber dadah, memantau trend pembuatan, dan menyokong pihak berkuasa dalam memerangi pengedaran dadah terlarang.

FORENSIC PROFILING OF METHAMPHETAMINE TABLETS BY ATTENUATED TOTAL REFLECTANCE-FOURIER TRANSFORM INFRARED SPECTROSCOPY

ABSTRACT

Illicit drug profiling is a vital aspect of forensic science, enabling law enforcement agencies to track drug distribution networks, identify adulteration trends, and enhance drug-related investigations. This study explores the application of attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy combined with principal component analysis (PCA) to chemically profile the methamphetamine tablets seized in Malaysia. In this study, a total of 161 methamphetamine tablet samples seized from the Kelantan region were analysed using ATR-FTIR spectroscopy. Subsequently, the ATR-FTIR profiles of these samples were subjected to PCA for clustering and discrimination. The results indicated that most samples contained high levels of adulterants, with caffeine being the most prevalent. The study also revealed that a significant proportion of the samples which is 157 samples (97.5%) contained less than 25% methamphetamine, suggesting the widespread adulteration practices aimed at increasing profit margins while maintaining stimulant effects. To conclude, the study demonstrates that ATR-FTIR spectroscopy, combined with PCA, offers a rapid, non-destructive, and cost-effective method for forensic profiling of drug samples. It allows forensic analysts to track drug sources, monitor manufacturing trends, and support law enforcement in combating illicit drug trafficking.

Chapter 1
Introduction

1.1 Background of the study

Forensic profiling of the drug also known as chemical fingerprinting consists of identifying, quantifying, and categorizing drug samples into groups. According to Ahmed et al. (2022), profiling is carried out to study the elucidation of synthetic pathways, identification of adulterants and impurities, and identification of a drug's geographic origin, specifically for plant-derived exhibits. Hence, drug profiling can be key in regulating illegal drugs from irresponsible parties in the country.

According to the World Health Organisation (2019), psychoactive drugs are defined as substances that when taken or injected into the body alter any of the mental processes like vision, concentration, consciousness, feeling, emotional state, and mood. Many psychoactive drugs are required to be produced, distributed, sold, or used for non-medical purposes by authourised channels only and strict laws impose or forbid the production, distribution, or sale of such psychoactive drugs. Dangerous Drug Act 1952 is an example of such a law which was enacted to regulate illegal drugs in Malaysia namely cannabis, opium, stimulant, and synthetic drugs. A psychostimulant is a class of drugs that enhance locomotor behavior by affecting the regulation of dopamine in the brain (Kropotov, 2014).

Methamphetamine is an example of a psychostimulant drug. Methamphetamine tablets popularly known as meth, speed, uppers, chalk, Yaba, rabbit, pep pills, or 'pil kuda'. They are one

of the most addictive and dangerous classes of drugs which have an impact on the brain. Methamphetamine tablets are small and usually contain adulterated caffeine. It is still popular among truckers and manual labourers to boost their physical performance. Methamphetamine is a powerful stimulant drug that affects the central nervous system in the form of tablets and powder. It increases energy, decreases appetite, and leads to euphoria lasting 6 to 12 hours. In addition, it also has dangerous side effects, such as nausea, high blood pressure, and hallucinations, which may damage the lungs, liver, and kidneys. It can be taken in different ways such as inhaling, smoking, and sniffing.

Methamphetamine is a type of amphetamine where the amino group of (S)-amphetamine has an added methyl group. Most of the illegal drugs (Methamphetamine) made in clandestine labs by using easily accessible chemicals have become a growing issue in Malaysian communities that leads to widespread addiction and associated problems. Based on the report *"Synthetic Drugs in East and Southeast Asia: Latest Developments and Challenges 2024"*, a new record revealed that 190 tonnes of methamphetamine were seized across East and Southeast Asia in 2023 (Vientiane, 2024). After a slight decline in 2022, methamphetamine seizures surged in 2023, reaching the highest level ever documented in the region. According to the UNODC (2024), authorities in East and Southeast Asia made the largest-ever seizure of methamphetamine, confiscating a staggering 1.1 billion tablets, which weighed a total of 98.3 tonnes, and the amount of crystal methamphetamine seized also hit a record, reaching 90 tonnes in 2023. Figure 1.1 illustrates those countries across the East and Southeast Asia, including Cambodia, China, Hong Kong, Malaysia, Myanmar, South Korea, Thailand, and Vietnam which have reported a rise in drug seizures during 2023.

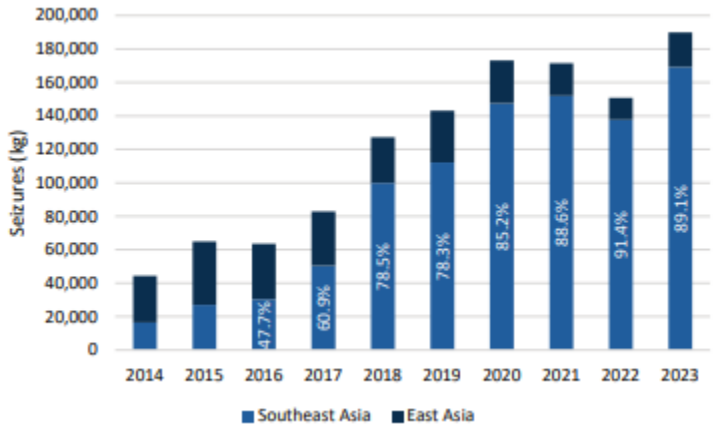


Figure 1 Methamphetamine seizures in East and Southeast Asia 2014-2023 (UNODC,2024).

1.2 Problem of statement

Methamphetamine tablets are commonly abused as methamphetamine throughout the globe, especially in the Southeast Asia including Thailand, Malaysia, Myanmar, Indonesia, and Laos. The ingredients and techniques used in the clandestine laboratory are manifold and vary historically in time and space (Stoneberg, Shukla, and Magness, 2017). To make the methamphetamine, manufacturers use precursor chemicals such as phenyl-2-propanone (P2P), ephedrine, and pseudoephedrine to manufacture methamphetamine. These tablets could have contained varying purity levels of methamphetamine by introducing adulterants during the manufacturing process to keep costs down, therefore often contain caffeine. According to the National Anti-Drugs Agency (NADA), Kelantan reported 645 cases of methamphetamine tablets addiction on 23 September 2023. Additionally, the Golden Triangle (Myanmar, Laos, and Thailand) produced more than 60 percent of Yaba or methamphetamine in the world (Ahad et al., 2017).

Thus, the forensic profiling of methamphetamine tablets is vital in reducing the cases and for forensic intelligence in Malaysia, particularly in the Kelantan state since Malaysia is close to the Golden Triangle. According to Hughes et al. (2013), the existing methods for identification and quantification are labour-intensive and time-consuming, taking significant time (more than a month) from submission before results are delivered. Most of the research focuses on the analysis of methamphetamine using gas chromatography-mass spectrometry. Since changing the production of methamphetamine may produce new impurity patterns; tracking illegal labs, learning new production methods, following trafficking routes, and knowing where the methamphetamine is coming from are important. Serving as a non-destructive technique, this study aims to chemically profile the methamphetamine tablets seized in Kelantan state using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy followed by chemometric method.

1.3 Aim and Objectives

The study was aimed to profile the methamphetamine tablets through ATR-FTIR spectroscopy. The two objectives set in this study were as follows:

i. To evaluate ATR-FTIR profiles of methamphetamine tablets.
ii. To compare the ATR- FTIR profiles of the seized methamphetamine through the chemometric method.

1.4 Significance of the study

The study aids forensic analysts in comparing the methamphetamine tablets from the various seizures through a non-destructive technique. The applied technique also allows for quick and low-cost determination of the ATR-FTIR profiles of methamphetamine tablets, subsequently tracking the possible source and composition of these samples. If the forensic analysts could determine the source of the drugs, the drug dealer's operation can also be studied thoroughly. For instance, it may provide valuable insight into how drugs work such as the manufacturing process and adulterants introduced to the illicit tablets. It plays an important role in tracking drug distribution and identifying the trafficking of drugs in Malaysia. It also fosters better investigations of drug-related crimes in the long run.

1.5 Scope of the study

All the samples were supplied by the Department of Chemistry Malaysia (Kelantan state). In this study, the tested samples were those samples submitted to the forensic laboratory for 26 months from July 2017 and August 2019. This study only focused on the qualitative determination of methamphetamine without quantifying the amount of active drug substance in the samples.

Chapter 2
Literature review

2.1 Methamphetamine

According to Vearrier et al. (2012), the history of methamphetamine began to develop as an amphetamine derivative, in which it quickly became a popular medication during the 1940s and 1950s, prescribed for a variety of indications. The popularity of methamphetamine tablets has gone through ups and downs over the years, but by the late 1980s, its illegal production and use were growing rapidly in Asia (UNODC, 2024). In 2016, it was estimated that over 35 million people around the world use meth and similar substances based on the United Nations Office on Drugs and Crime World Drug Report (Richards, et al, 2019). Methamphetamine supply was rapidly increasing in Asia, which is the largest market for amphetamine-type stimulants (ATS) and methamphetamine seizures have tripled, reaching 36 metric tonnes in the past five years (Akara, 2014). In the past years, the region of Golden Triangle (Myanmar, Laos, and Thailand) produces more than 60 percent of Yaba or methamphetamine in the world (Ahad et al., 2017).

Yaba, often called “Nazi speed” or “the madness drug,” is a synthetic illegal drug widely abused in parts of the world, especially in Thailand, Myanmar, Sri Lanka, and Bangladesh (Mukherjee, et al, 2022). Yaba is typically found in red tablet form, often with added artificial flavours. Methamphetamine is available both as tablets and in crystal form. The most common way people use it is by taking it orally. However, the drug abuse user may also crush the tablets and inhale the fumes by heating them on foil which is the same method as how heroin is "chased." Yaba pills are considered more dangerous than other stimulants because a large portion of their amphetamine content remains unchanged in the body (Mukherjee et al., 2022).

2.2 Adulterant of methamphetamine

An "adulterant" is a substance with active ingredients, and a "diluent" is an inactive filler (Cole et al., 2010). In this study, the term "adulterant" collectively refers to substances that are mixed into illegal drugs during the process of selling and distribution. Adulterants are usually other psychoactive substances such as caffeine or paracetamol. These are much cheaper than the main drug, and when mixed with the drug, it has similar or complementary effects. The use of adulterants can help to conceal the fact that the original substance has been diluted. Substances that are not psychoactive are more formally known as 'diluents' (Coomber, 1997). Sugar compounds like lactose, dextrin, glucose, sucrose, and sorbitol, along with inorganic compounds such as silicates and magnesium salts, can serve as diluents (Haywood and Glass, 2011).

Yaba tablet is a highly addictive combination of methamphetamine and caffeine. A study from Junkuy et al., (2014) stated that methamphetamine (MA), is customarily mixed with caffeine and sold in tablet form under the general name of “Yaba” (the literal translation from Thai is crazy drug). Chemical analysis of Yaba tablets seized by Thai police revealed an average content of 21.76 ± 6.39 mg% MA and 62.43± 9.15 mg% caffeine (Adam et al., 2005). There was also support from another study by Rahman Rezvi (2019) stated that methamphetamine synthetically produced pills containing 25 to 35 mg of methamphetamine and 45 to 65 mg of caffeine.

2.3 Structure of methamphetamine

Drug profiling is a scientific instrument to discover the synthesis routes, the sources of supply, trafficking routes, and links between seizures that help drug enforcement agencies combat organised drug crime. Thus, it is important to understand the structure of methamphetamine to make a profile.

IUPAC defines the systematic name of methamphetamine namely (2S)-N-methyl-1-phenylpropan-2-amine and known as methamphetamine, N-methylamphetamine, methylamphetamine, and desoxyephedrine. It is a psychostimulatory drug that belongs to the phenethylamine and amphetamine group, in which the amino group of (S)-amphetamine has a methyl group attached (Pubchem, 2000). The molecular formula of methamphetamine is C10H15N, and the chemical structure is shown in Figure 2.1.

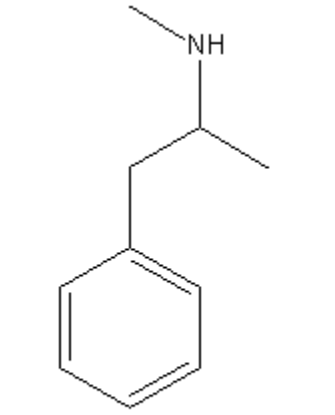


Figure 2.1 Chemical structure of methamphetamine.

Methamphetamine and its precursor chemicals such as ephedrine and pseudoephedrine (PEP) are chiral compounds due to their asymmetric carbon atoms (Inoue,et al, 2008). Methamphetamine consists of two chemical subgroups which are dextromethamphetamine and levomethamphetamine in their pure amine forms (Yasaei and Saadabadi, 2023). The highly abused form is the dextromethamphetamine (d-isomer) form and generally found in illicit preparations. However, the detection of abuse is difficult because using over the counter and prescription medications can give a positive result, but the analytical method is not capable of differentiating between d- and l-enantiomers. According to Alagandula and Carroll (2019), the chiral separation of d- and l-methamphetamine and their metabolites d- and l-amphetamine can help determining whether the source was licit or illicit, however, the chiral columns can be expensive, and may necessitate a dedicated instrument, and are not as broadly useful as ubiquitous C18 columns used in liquid chromatography. Figure 2.2 shows the chemical structures of d- and l- methamphetamine enantiomers.

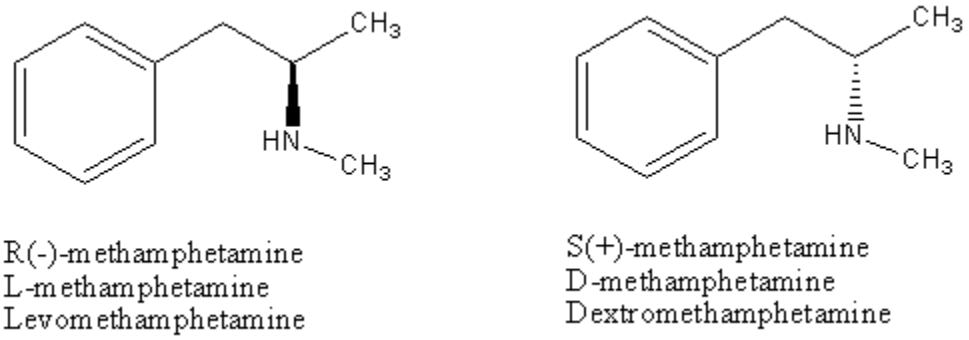


Figure 2.2 Chemical structures of Levomethamphetamine and dextromethamphetamine

2.4 Synthesis routes of methamphetamine

Synthetic drugs of abuse are a large and diverse group of psychoactive substances that have been engineered and synthesised in a chemical laboratory. Communities abuse and recreational use of these substances that initially developed for use in pharmacological research (Tamama, 2020). An illegal drug is an assemblage of constituents carrying information about the synthetic route, condition-specifics, reagents, adulterations during trafficking, synthetic batches, and sometimes the chemical process level of the cooks (purity) (Onoka et al., 2020).

The clandestine synthesis of methamphetamine has relied on three primary precursors for quite a long time, namely ephedrine, pseudoephedrine, and 1-phenyl-2-propanone (P2P) (Onoka et al., 2020). According to Figure 2.3, two major synthetic groups can be distinguished the syntheses starting from P2P followed by the Leuckart method and reductive animation yields racemic methamphetamine, and the second is the syntheses using l-ephedrine or d-ephedrine as the starting materials, the former gives the methamphetamine, which is more potent than racemic for the central nervous system (Inoue et al, 2008). The other synthesis routes of methamphetamine include the Nagai, Moscow, Rosenmund, Birch reduction, Hypo, and Emde methods with chloroephedrine as intermediate during the manufacturing process.

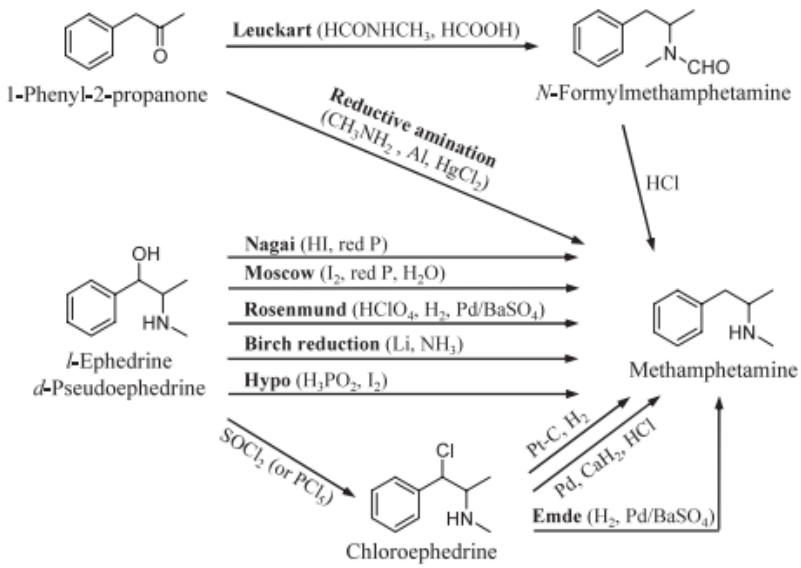


Figure 2.3 The synthesis routes of methamphetamine (Inoue et al, 2008).

The most used method for the clandestine manufacture of methamphetamine due to its simplicity is called the Leuckart reaction (Frank M. Hauser et al., 2018). The Leuckart reaction is a reductive alkylation. In this reaction, the alkylating agents such as P2P and formic acid are used along with an amine like ammonia or methylamine to react. These formylation steps yield formylamphetamine which is then hydrolysed in aqueous acid to the corresponding amphetamine (Stojanovska et al., 2013). A widely used method for performing reductive amination includes the reactions of a ketone with an amine, such as ammonia or methylamine, forming a hemiaminal intermediate (Stojanovska et al., 2013). On the other hand, this hemiaminal is reduced with reducing agents such as sodium cyanoborohydride, sodium borohydride, or aluminum in conjunction with a mercury chloride catalyst to generate the corresponding ATS (Stojanovska et al., 2013). The detection of N-formylmethamphetamine (FMA) is significant in any

methamphetamine drug profile, since FMA is an intermediate in the synthesis of methamphetamine by the Leuckart reaction (Barnes et al., 2019).

2.5 Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy is a rapid, non-destructive technique that offers the benefits of quick analysis and ease of use (He et al., 2020). Samples are crushed and subsequently measured by pressing them directly on the crystal surface (Custers et al., 2015). Screening counterfeit medicines with ATR-FTIR can be convenient and requires low sample preparation (Custers et al., 2015). Hence, confirmatory identification of unknown samples can be made by the selective absorption of infrared (IR) radiation with ATR- FTIR spectroscopy (Dash et al., 2023).

FTIR spectroscopy is a popular routine analytical technique used to study organic molecules. In the mid-IR region (4000 cm^{-1} to 400 cm^{-1}), there is a large abundance of information regarding the structure of functional groups within the analyte (Hughes et al., 2013). A big attribute of IR is its ability to identify a chemical compound by absorbing specific wavelengths that are unique to that compound because of the molecular bonds that existed in the sample. Each unique molecular structure produces a unique infrared spectrum, and spectroscopy has great utility in forensic analyses, especially involving powder or crystalline samples (Dash et al., 2023). The second application of FTIR allows it to be performed quantitatively where the more energy absorbed by certain wavelengths; the more bonds are absorbing the energy (Hughes et al., 2013). Hence, greater energy absorption occurs with larger analyte concentrations.

Attenuated total reflection (ATR) is an important molecular sampling accessory for the effective measurement of micro areas on irregular and curved sample surfaces (He et al., 2020). Broadening the applications of infrared spectroscopy substantially simplifies sample preparation and processing. Achieving forward progress in forensic drug analysis depends largely on advancing it by developing simple and inexpensive analytical methods.

According to research by Shahzad Munawar et al. (2024), they studied the detection of methamphetamine using ultraviolet-visible spectroscopy (UV-Vis), FTIR, and gas chromatography mass spectroscopy (GC-MS) techniques. The authors reported that the FTIR spectrum showed 88% methamphetamine while GC-MS showed 85% methamphetamine. A study by Hughes et al. (2013) analysed a total of 96 illicit drug through ATR-FTIR. The tested samples were found to contain methamphetamine ranging from 0.1% to 78.6% through the GC-MS. In the study, chemometric techniques were also applied on the sample profiles in which the partial least squares (PLS) method was shown to be the most effective for determining the concentration range of drug substances in the tested samples.

He et al. (2020) assessed the effectiveness of ATR-FTIR combined with chemometric methods for the rapid forensic classification of heroin hydrochloride, methamphetamine hydrochloride, ketamine hydrochloride, and their five common additives namely caffeine, glucose, phenacetin, starch, and sucrose. The findings in their research identified that repetitive and interfering data in original spectrum data could be eliminated by PCA and factor analysis. The Bayes discriminant analysis model was suggested as a useful and practical method for classifying the target drugs of abuse. Riyanto and Nas (2016) also studied the validation of analytical methods for the determination of methamphetamine using FTIR spectroscopy and concluded that validating

analytical methods for determining methamphetamine using FTIR has proven to be highly effective.

Previous studies demonstrated the utility of FTIR to analyse methamphetamine and other illicit drug for forensic investigations. Solid-form drugs could be characterised by FTIR due to their practical and cost-efficiency advantage over the other techniques. Additionally, FTIR has become a particularly reliable technique in forensic analysis due to the wide availability of advanced software and exhaustive spectral libraries.

2.6 Principal component analysis

Chemometrics is a chemical discipline that applies mathematics, statistics, and formal logic (Deconinck et al., 2022). The overall goal is to develop efficient quantitative techniques to assist in designing or selecting the most effective experimental procedures to address the question, extracting maximum relevant chemical information from the analysis of chemical data, and gaining chemical insights into the system (Deconinck et al., 2022).

The application of spectroscopy and chemometrics integration is common practice across many fields including illicit drug analysis. These applications tend to remain within scientific research. When spectroscopic techniques (FTIR and Raman) are combined with chemometric methods, they could be more effectively utilized in drug and forensic analysis (Deconinck et al., 2022). Such instruments like FTIR is usually used with a spectral library, but chemometric techniques are essential in extracting information like bringing about a full characterisation of samples including adulteration. An example of a chemometric technique is PCA.

PCA is a ubiquitous statistical method used to identify and reframe the data through linear mapping. It is a multivariate statistical technique to insight a data table where observations are composed of a few interrelated quantitative dependent variables (Abdi and Williams, 2010). This technique reduces a set of possibly correlated variables into a set of principal components, which are uncorrelated variables. Exploratory analysis of samples was achieved PCA can revealed a trend of similarity and differences among the samples by reducing data dimensionality (Stevanović et al., 2020). PCA attempts to find the key information from the data table and as such represents this as a set of new orthogonal variables (known as principal components) (Abdi and Williams, 2010). It also aims to show the patterns of similarity among observations and variables as points on maps.

According to research by Rodrigues et al. (2013), they assessed the spectra of 91 samples of cocaine that were collected using ATR-FTIR spectroscopy and utilised an exploratory PCA model where their findings are lower-purity cocaine samples represented 42.8% of PC1 distribution differences because they commonly contained additives including lidocaine, caffeine, and benzocaine, and 20.2% of PC2 highlighted the distinction between the two main forms of cocaine which are base and salt. Stevanović et al. (2020) proposed in their studies to develop a reliable and rapid method for classifying illicit heroin samples and determining the concentration range of heroin in these samples by ATR-FTIR spectroscopy in conjunction with chemometric techniques such as PCA, cluster analysis, and PLS. The findings of the studies identified that the clustering method effectively demonstrated the ATR-FTIR technique's ability to categorise samples into separate groups and demonstrate spectroscopy linked to chemometrics techniques representing a powerful combination that benefits forensic work and drug control facilities. According to Hughes et al. (2013), ATR-FTIR spectroscopy was also used to analyse 96 illicit drug

seizures containing methamphetamine with chemometric techniques, suggesting the efficiency of PLS. In another study by Teoh et al. (2022), ATR-FTIR spectroscopy was used to analyse four sedative-hypnotics, diazepam, ketamine, nimetazepam, and xylazine followed by PCA and Linear Discriminant Analysis (LDA) on the resulting ATR-FTIR profiles. The author reported that the proposed PCA score-LDA model had allowed for a 100% accurate classification and useful in detecting and discriminating sedative-hypnotics, including those that had been previously spiked into a beverage. The study had allowed for comparison and decomposition to enable their detection and discrimination.

From the previous studies above, it is shown that the combination of FTIR and PCA is a fast and nondestructive analytical tool and has great value in aiding criminal investigations. It is proved that the joined application of spectroscopy and chemometrics can be extremely convenient and useful for forensic and drug control laboratories (Nataša et al., 2020). In addition, a similar technique has recently been applied in other forensic areas, including discriminating against ballpoint pen inks from spray paints (Rodrigues et al.,2013). The studies on methamphetamine by using ATR-FTIR coupled with PCA had limited exploration, where most research is about other illicit drugs such as heroin and cocaine.

CHAPTER 3
METHODOLOGY

3.1 Introduction

A drug profiling was carried out to examine illicit methamphetamine tablets, focusing on their chemical characteristics and identifying the main and minor components, including adulterants in each sample.

3.2 Standards and chemicals

Standards and chemicals utilised are demonstrated as follows:

i. 99.7% purity of methamphetamine (Department of Chemistry Malaysia)
ii. 99.2% purity of caffeine (Sigma-Aldrich, St. Louis, MO, USA)
iii. 2-propanol (Merck, Whitehouse Station, NJ, USA)
iv. Methanol (Supelco, Bellefonte, PA, USA)
v. Sugars including fructose, lactose and mannitol (Merck, Whitehouse Station, NJ, USA)
vi. Starch (Sigma-Aldrich, St. Louis, MO, USA)

3.3 Instrumentation

The instrument used in the was Bruker FTIR Alpha II, equipped with a zinc selenide ATR sampling accessory from Bruker Corporation (Billerica, MA, USA).

3.4 ATR-FTIR analysis of the samples

This study analysed 161 illicit methamphetamine tablet samples seized over 26 months between July 2017 and August 2019. These samples were submitted to the Department of Chemistry Malaysia by the Royal Malaysian Police. Due to limited quantities, at least two tablets from each seizure were retained, depending on the size of the confiscated materials. Each sample was carefully recorded and labeled with a unique identification number for this study, while the actual case numbers were kept confidential for security and privacy reasons.

3.4.1 Preparation of standard and illicit drug samples

The drug samples were carefully ground into a fine powder using a pestle and mortar. The pestle and mortar were cleaned using methanol before grinding another sample to ensure no cross-contamination between samples. Then, the powdered samples were placed in labeled vials separately for storage. The vials were labeled with P001 until P161, totaling 161 samples. No preparation was needed for the standard substances because the standards already appeared in powder form. These standards covered methamphetamine, ephedrine, caffeine, and several sugars like fructose, lactose, and mannitol were used to create reference ATR-FTIR spectra. These spectra

served as a comparison for analyzing the unknown compositions of the illicit drug samples. The reason these standards are used for reference is because they are common adulterants used in illegal drugs.

Mixed samples were prepared with different ratios of the active compound and adulterants to examine the effect of mixture composition on ATR-FTIR profiles. Hence, based on the literature review, the adulterant selected for this study was caffeine. Methamphetamine to caffeine ratio was set at 100%, 25%, 10%, and 0%, with precise weight-to-weight measurements. For example, to prepare a mixed sample with 25% methamphetamine and 75% caffeine, 10% methamphetamine and 90 % caffeine, 0% methamphetamine and 100% caffeine and 100% methamphetamine and 0% caffeine.

3.4.2 Analysis of powdered samples using ATR-FTIR

Around 10 milligrams of each sample and standard were placed directly onto an ATR crystal without any sample preparation. Before each test, the crystal was cleaned with 2-propanol rubbing using cotton buds. Before analysing each sample, a background measurement was done to eliminate any interference in the ATR-FTIR spectra. The test was conducted at a consistent room temperature of around 25 ˚C.

Absorbance was taken across a wavelength range of 4000 to 600 cm^{-1}, with a resolution set at 4 cm^{-1}. Each sample was scanned 16 times during the analysis. The spectra were captured, and the system was operated using OPUS 7.0.122 software (Bruker Corporation, Billerica, MA, USA). The data was saved in the form of CSV files. Both standard and drug samples spectra were recorded in duplicate for comparison and evaluation. To ensure precision of the analytical method,

repeatability was tested by analysing a representative sample three times while reproducibility was verified by testing the same sample over three consecutive days. Absorbance values were recorded at each data point for interpretation and comparison. All the ATR-FTIR profiles were interpreted and the functional groups that contributed to the absorbance within the profiles were determined.

3.5 Principal component analysis

The data from regions with significant variations (1800–600 cm^{-1}) were used for drug profiling. These regions are known as fingerprint regions and functional group regions in which they are important for statistical analysis. The ATR-FTIR spectra was exported to Microsoft Excel® (Redmond, WA, USA) for data treatment such as baseline correction and normalisation. Then, these data were transported to Minitab® 20 software (Minitab Inc., State College, PA, USA) to cluster or discriminate the illicit methamphetamine samples. PCA was used to group the samples based on ATR-FTIR spectra, exploring their similarities and differences with standards and methamphetamine-adulterant mixtures. Variations among the illicit drug samples were assessed based on their compositional profiles.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Introduction

A total of 161 illicit methamphetamine tablet samples were analysed in this study. The ATR-FTIR profiles of the seized samples were obtained, compared, and differentiated among them. All the findings were described and discussed.

4.1 ATR-FTIR SPECTRA

Drug samples were analysed using ATR-FTIR spectroscopy to establish their profiles, determine the structure, compare with the methamphetamine standard, and distinguish between the seized samples. The ATR-FTIR spectra was generated through analyses and compared among the seized samples.

4.1.1 Repeatability and reproducibility test

Tests for repeatability and reproducibility were performed before determining and comparing the samples. The repeatability study was carried out using ATR-FTIR profiles obtained on a representative sample (P100) from the same seizures as illustrated in Figure 4.1. Using the same sample, the ATR-FTIR profiles were generated thrice. The ATR-FTIR profiles of P100 are found to be repeatable, suggesting the production of consistent results through repeating analyses within a short duration of time.

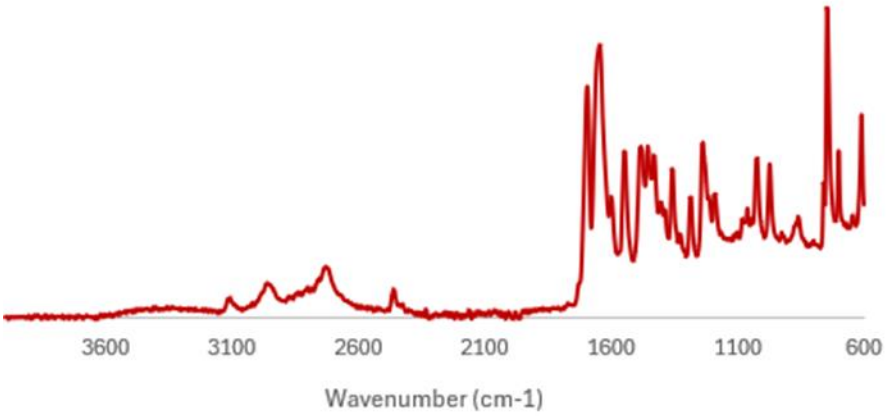
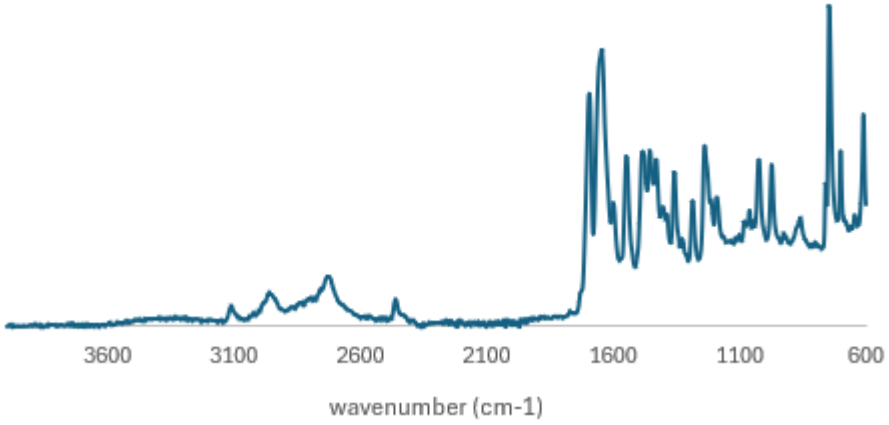
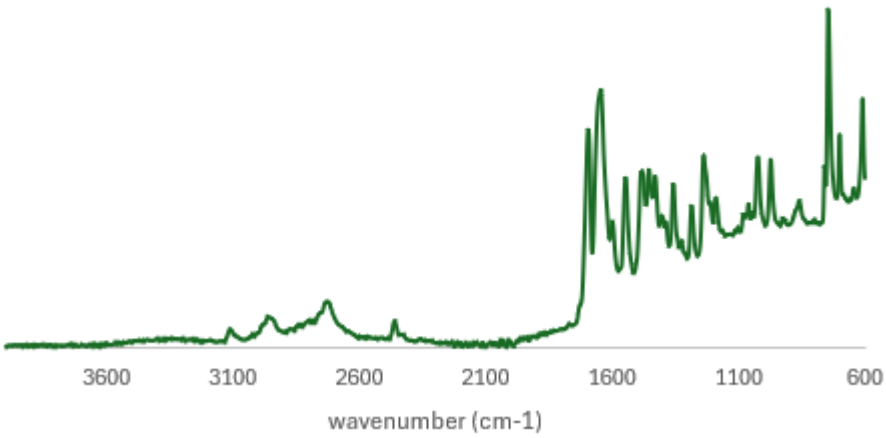
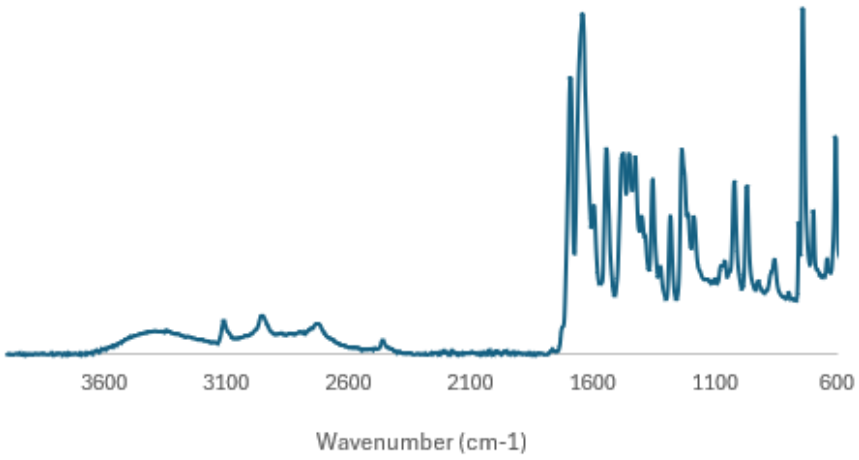
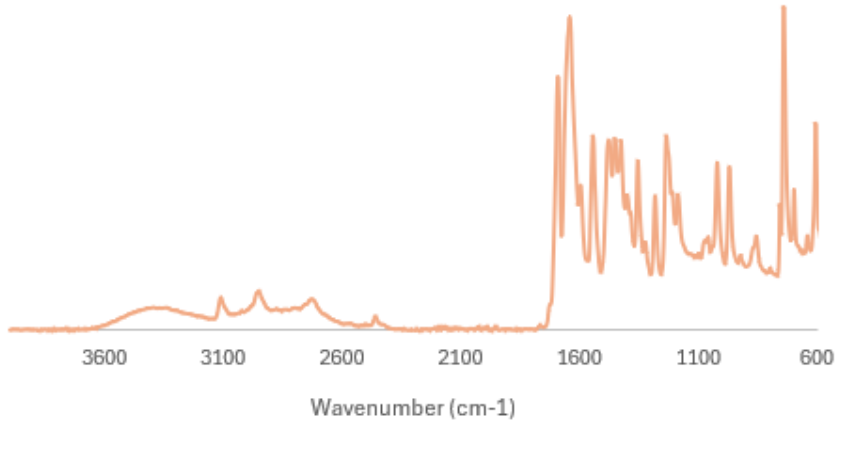
Sample P100(A)	
Sample P100(B)	
Sample P100(C)	

Figure 4.1 Comparison of ATR-FTIR profiles of sample P100 for repeatability test.

For the reproducibility test, the ATR-FTIR spectra of the same sample were found to have no differences in spectra obtained three times over three consecutive days. The representative spectra (P142) from the reproducibility test is shown in Figure 4.2. These findings indicated that the ATR-FTIR technique had yield consistent results across multiple analyses even though over three days duration.

P142 (DAY 1)	
P142 (DAY 2)	

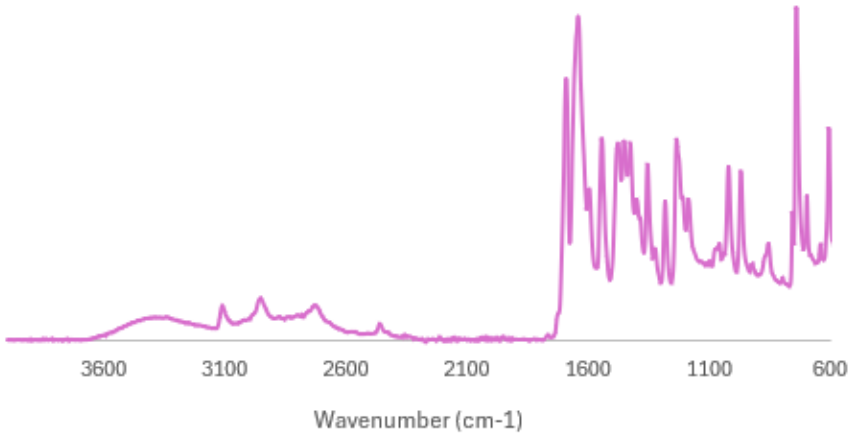
P142 (DAY 3)	

Figure 4.2 Reproducibility test of sample P142.

Figure 4.3 shows the ATR-FTIR spectra of methamphetamine standards and various adulterants including caffeine, ephedrine, and various sugars that may be added during the manufacturing of methamphetamine tablets. Through visual observation, each profile was found to be different due to the varying chemical structures that made up the substance. Even from the sugar group which could have a similar composition, their ATR-FTIR profiles also appeared to be slightly different, probably because of the functional groups that existed in the chemical structure.