QUANTIFICATION OF METHAMPHETAMINE IN SEIZED TABLETS BY GAS CHROMATOGRAPHY- FLAME IONISATION DETECTOR

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QUANTIFICATION OF METHAMPHETAMINE IN SEIZED TABLETS BY GAS CHROMATOGRAPHY- FLAME IONISATION DETECTOR

by

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CERTIFICATE

This is to certify that the dissertation entitled "Quantification of Methamphetamine in Seized Tablets by Gas Chromatography- Flame Ionisation Detector" is the bona fide record of research work done by Nor Syafiatul Fitri binti Roslan during the period of October 2024 to February 2025 under my supervision. I have read this dissertation and that is my opinion in it conforms to acceptable standards of scholarly presentation and is gully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Science (Honours) (Forensic Science).

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise sated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purposes.

…… ……

(NOR SYAFIATUL FITRI BINTI ROSLAN)

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LIST OF SYMBOLS

%	Percentage
m	Meter
mm	Millimeter
µm	Micrometer
°C	Degree Celcius
mL/min	Milimeter per minute
mg/L	Milligram per litre
µg/mL	Microgram per mililitre
µg/L	Microgram per litre
µL	Microlitre
±	Plus-minus
°C/min	Degree Celcius per minute
min	Minute
R^2	Correlation coefficient
ppm	Parts per million

LIST OF ABBREVIATIONS

ADHD	Attention deficit hyperactivity disorder
AM	Amphetamine
ATS	Amphetamine-type stimulant
FDA	U.S. Food and Administration
FID	Flame ionisation detector
GC	Gas chromatography
GC-MS	Gas chromatography- mass spectrometry
HP-5MS	(5%-phenyl)-methylpolysiloxane
ICH	International Council for Harmonisation
LOD	Limit of detection
LOQ	Limit of quantification
MAMP	Methamphetamine
MBDB	N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine
MDA	3,4 methylenedioxyamphetamine
MDEA	3,4-Methylenedioxy- N-ethylamphetamine
MDMA	3,4-methylenedioxymethamphetamine
NADA	National Anti-Drugs Agency
OAS	The Organisation of American States
PBA	Phenylbenzylamine
PFPA	Pentafluoropropionic anhydride
PTFE	Polytetrafluoroethylene
RSD	Relative standard deviation
UNODC	United Nations Office of Drugs and Crimes
S/N	Signal-to-noise

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QUANTIFICATION OF METHAMPHETAMINE IN SEIZED TABLETS BY GAS CHROMATOGRAPHY- FLAME IONISATION DETECTOR

ABSTRAK

Metamfetamin, adalah dadah yang berfungsi sebagai perangsang yang menjejaskan sistem saraf pusat. Dadah tersebut telah menjadi kebimbangan di seluruh dunia kerana panyalahgunaan yang meluas dan memberi kesan buruk terhadap kesihatan badan. Kajian ini berfokus untuk kuantifikasi sampel metamfetamin yang dirampas di Kelantan, Malaysia, menggunakan kromatografi gas terganding dengan pengesan pengionan nyalaan (GC-FID). Kuantifikasi metamfetamin adalah penting untuk proses penghakiman, kerana kuantiti dadah secara langsung mempengaruhi hukuman undang-undang. Dalam kajian ini, pengasingan kromatografi dilakukan menggunakan suatu turus HP-5 MS (30 m x 320 μm x 0.25 μm) dengan suhu program yang ditetapkan pada 70 °C selama 2 minit, diikuti dengan kenaikan suhu sebanyak 20 °C/min hingga 280 °C selama 2 minit menggunakan gas nitrogen tulen sebagai gas pembawa pada kadar aliran 1.0 mL/min. Kaedah ini didapati memuaskan dan berjaya digunakan untuk mengkelaskan sampel metamfetamin dengan masa analisis selama 14.5 minit. Kaedah GC-FID telah disahkan dengan menilai kelinearan, had pengesanan (LOD), had kuantifikasi (LOQ), ketepatan dan kejituan. Lengkung kalibrasi berjulat 1.95-1000 μg/mL, mewujudkan pekali korelasi 0.9976. Had pengesanan dan had kuantifikasi masing-masing adalah 0.293 μg/mL and 0.975 μg/mL. Ujian kejituan menunjukkan nilai %RSD di bawah 2%. Ujian ketepatan menunjukkan peratusan pemulihan antara 85–106%. Analisis kuantifikasi bagi 155 sampel metamfetamin menunjukkan ketulenan dengan julat antara 0.5-30.6%. Kaedah

mudah ini adalah sesuai untuk analisis rutin forensik, terutamanya dalam penentuan kuantiti metamfetamin dalam sampel dadah yang dirampas.

QUANTIFICATION OF METHAMPHETAMINE IN SEIZED TABLETS BY GAS CHROMATOGRAPHY- FLAME IONISATION DETECTOR

ABSTRACT

Methamphetamine, is a drug function as a stimulant that affect the central nervous system. It has become a significant global concern due to its widespread abuse and dangerous health effects. This study focused on the quantification of methamphetamine in seized tablet samples from Kelantan, Malaysia, using gas chromatography coupled with flame ionisation detector (GC-FID). Methamphetamine quantification is critical for judicial processes, as the drug's quantity directly affects the severity of legal charges. In this study, chromatographic separation was performed on a HP-5 MS column (30 m x 320 μm x 0.25 μm) with a temperature programme of 70 °C for 2 min, followed by a ramp up at 20 °C/min to 280 °C for 2 min with purified nitrogen as the carrier gas at a flow rate of 1.0 mL/min. The method was found to be satisfactory and successfully applied for quantification of methamphetamine samples with analysis time of 14.5 min. The GC-FID method was validated by assessing its linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy. Calibration curves ranged 1.95 - 1000 μg/mL, established a correlation coefficient of 0.9976. Limit of detection and limit of quantification were 0.293 μg/mL and 0.975 μg/mL respectively. The precision test showed %RSD value below 2%. The accuracy test showed percentage recovery between 85 - 106 %. Quantification analysis of 155 methamphetamine samples indicated purity with range of 0.5 - 30.6%. This simple method was suitable for forensic routine analysis, especially in quantifying the methamphetamine from seized samples.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Evolution of illegal substance abuse from opiate-type drugs to psychostimulants had been occurred globally over the years. Methamphetamine, $C_{10}H_{15}N$ is a synthetic stimulant that is addictive and can cause considerable health adversities. The U.S. Food and Administration (FDA) has approved methamphetamine as a second-line treatment for treatment of attention deficit hyperactivity disorder (ADHD) and for short-term treatment of obesity. However, most methamphetamine is illicitly manufactured which is better known as a recreational drug (Dhamir et al., 2023).

Methamphetamine has become a dynamic issue of global concern. According to The Organisation of American States (OAS), methamphetamine is the most manufactured amphetamine-type stimulant (ATS) in the world. ATS are synthetic drugs that do not require the extraction of active constituents from plants that have to be cultivated such as heroin and cocaine and therefore can be made almost anywhere (Stoneberg et al., 2017). This adaptability makes methamphetamine production easier and more widespread, contributing to its prominence in global drug markets.

In Malaysia, methamphetamine is classified as a controlled drug under Dangerous Drug Act 1952 that regulates the importation, exportation, manufacture, sale and use of the drugs. According to the National Anti-Drugs Agency (NADA), crystal methamphetamine was mostly abused by drug addicts in Malaysia (2020) as the drugs are relatively cheap and easily accessible. It is a powerful, highly addictive stimulant that affects the central nervous system by triggering the heightened

production of dopamine in the brain and remains in the brain's synapse for long periods after use (Vincent, 2021). When high levels of dopamine are released, the pleasurable effects happened. As with many stimulants, users often misuse methamphetamine in a "binge and crash" pattern where users often try to maintain the high by taking more methamphetamine before the first dose wears off, extending the stimulant effects (Davis, 2023). Users may use methamphetamine continuously for several days, avoiding sleep and food while taking the drugs.

Street name for methamphetamine also known as ice, crystal meth or shabu. In either crystal, pill or powder form, they can be easily dissolved in water or alcohol. Methamphetamine was developed from its parent drug, amphetamine that can be injected, smoked, snorted or ingested orally. When methamphetamine is injected or smoked, it enters the bloodstream and brain rapidly thus producing an immediate and intense rush or euphoria to its users (Davis, 2023). Other immediate effects include increased activity and decreased appetite. Adverse effects for long term consumption include mental health disorder, effects on cognition and health such as stroke, myocardial infection, seizures and death (National Institute on Drug Abuse, 2024).

Quantification of illicit drugs, where measuring their amount or concentration plays a crucial role in addressing illegal drug abuse and drug trafficking. This process is essential for law enforcement and judicial process against suspects of a drug-related crime. The amount of illicit drug can never be compromised because the court of law relies mostly on analytical results to make fair judgement as the amount of the substance directly impacts the severity of the charges, thus an accurate quantification is needed to deter criminal activity.

When identifying an illicit substance, it is important to adhere to well accepted, and reliable methodologies to ensure that only robust, accurate and defensible analytical results are obtained (Donovan et al., 2023). Gas chromatography (GC) combined with flame ionisation detection (FID) has become a standard technique for analysing volatile compounds in complex mixtures. Its application in forensic science, particularly in the detection and quantification of illicit drugs, has proven essential. This is particularly true for methamphetamine, a potent stimulant commonly associated with criminal activity. In this context, GC-FID provides a reliable method for both qualitative and quantitative analysis of methamphetamine in seized drugs. The synergy between GC and FID allows for high sensitivity and accuracy, making it a preferred method for forensic drug analysis.

1.2 Problem statement

The abuse of methamphetamine has become a growing concern globally, with significant increases in usage and distribution reported over the past several years. This escalating problem is evident through the rising quantities of methamphetamine seizure, serving as an indicator of the expanding market and its availability. Based on data from the United Nations Office on Drugs and Crime (UNODC), data show an upward trend in methamphetamine tablets seizure between 2017 and 2021. Though the amount of methamphetamine tablets declined slightly in 2022, it remains at a high level.

The drug is often distributed in tablet form and the methamphetamine content within these tablets can be varied. Variation in the composition of illicit drugs, particularly the active methamphetamine was reported based on the sources of these

drugs and the manufacturing pathway. It is important to accurately quantify the illicit drugs for prosecution purposes and thus, a designated method is required for an accurate and reliable method can be utilised for quantification purpose. As for this study, a previous validated method was available implementing the GC instrumentation. The method was adapted to quantify the content of methamphetamine and explore the variation among the tested samples.

1.3 Objectives

1.3.1 Main objectives

To quantify methamphetamine contained in seized samples by GC-FID.

1.3.2 Specific objectives

i. To validate the GC method used for analysis of methamphetamine in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy.

ii. To determine the purity of seized methamphetamine tablets.

iii. To explore the compositional variation among the seized methamphetamine tablets.

1.4 Significance of study

This study could contribute to more reliable and precise methods in quantifying methamphetamine. The refinement of analytical techniques can ensure accurate measurement of methamphetamine content in the seized samples. The ability to accurately quantify methamphetamine in seized tablets is vital, as the quantity of the drug directly impacts the severity of the charges. This study also focuses on providing

detailed information of the purity levels of methamphetamine tablets seized in Kelantan state, offering insight of the illicit manufacturing network besides quality and composition of methamphetamine tablets circulating in the region. By enhancing the precision of methamphetamine quantification methods and analysing the purity of seized samples, this study will improve enforcement efforts and contribute to the broader understanding of drug abuse as well as manufacturing patterns.

1.5 Scope of study

This study focused on the quantification of methamphetamine tablet samples seized in Kelantan. The determination of methamphetamine contained in each sample was made based on the comparison of the retention time of standard.

CHAPTER 2

LITERATURE REVIEW

2.1 Principle and mechanism of GC-FID

GC is a chromatographic technique that is especially effective for separating volatile compounds. It relies on the movement of a sample through a column under a controlled temperature, with a carrier gas helping to push the sample through the column. Firstly, the sample is introduced into the GC system through an injection port. It is vapourised immediately due to the high temperature of the injector, ensuring it enters the column in gaseous state. Then, a carrier gas usually helium, nitrogen or hydrogen gas transports the vapourised sample through the column. The carrier gas is inert and does not interact chemically with the analytes.

The sample interacts with the stationary phase inside the column, allowing the components of the sample to separate based on their volatility and affinity for the stationary phase. Less volatile compounds or those with greater affinity for the stationary phase retained longer, while more volatile compounds elute faster. Following this separation, a detector analyses the components when they exit the column. In GC-FID, the flame ionisation detector (FID) is used, which involves burning the sample in a flame, ionising the compounds, and detecting the ions produced. The resulting signal is proportional to the number of ions, thus providing information about the concentration of the analytes present in the sample (Costa et al., 2024). Figure 2.1 illustrates the component of GC-FID.

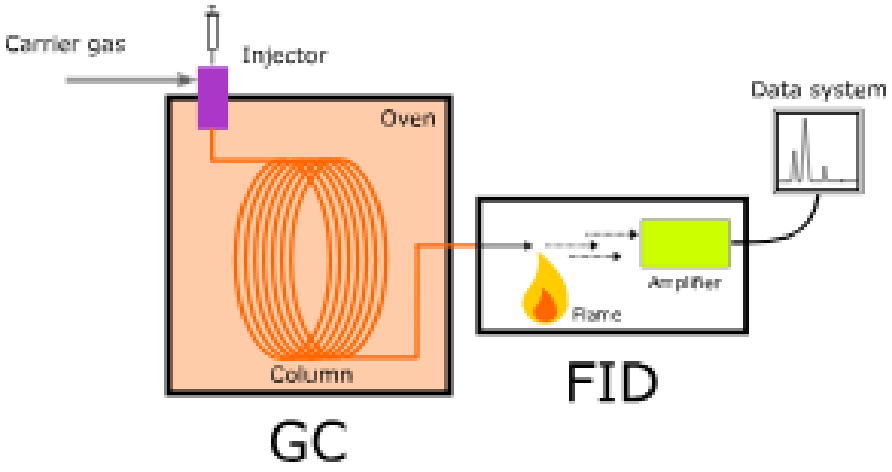

Figure 2.1 Overview diagram of GC-FID (Source: Pyrenessences, 2024)

The use of FID is particularly advantageous for the analysis of organic compounds like methamphetamine, as it provides high sensitivity and selectivity. Methamphetamine, a volatile compound, is readily detectable using this method, making it highly suitable for forensic drug analysis. FID is also known for its low detection limits and stability, further contributing to the reliability of the results in a forensic setting (Moylan et al., 2021, Ravindran et al., 2021).

2.2 Column separation and temperature programming

One of the most critical aspects of optimising a GC-FID method is selecting the appropriate chromatographic column. The column serves as the medium for separating the different compounds in a sample, and its properties, such as the stationary phase and dimensions, greatly influence the resolution and separation efficiency. Premathilaka et al. (2024) used a (5%-phenyl)-methylpolysiloxane (HP-5MS) column for their GC-FID method, which is a widely used column for separating volatile organic compounds due to its ability to provide good separation efficiency and resolution. The column, with its 30m x 0.25mm x 0.25µm dimensions, allows for the efficient separation of methamphetamine from other potential contaminants in seized tablets, which often

contain various excipients and impurities that can interfere with analysis. The selection of this column ensures that methamphetamine could be effectively separated from these matrix components, providing a clearer signal for its detection.

Differ from Premathilaka et al. (2024) and Rajagopal et al. (2024), Mitrevski and Zdravkovski (2004) used Supelco SPB-50 capillary column (15 m x 0.32 mm i.d.; 0.5 mm). Supelco SPB-50 thickness made up of 50% of phenyl and 50% dimethylysiloxane. It can separate and analyze a broad range of amphetamines, including methamphetamine without derivatisation. The higher phenyl content improved separation of polar amphetamines with reduced peak tailing, making it suitable for drug tablets with mixed compounds. While SPB-50 is a medium polarity column, HP-5MS is a low polarity column with 5% phenyl and 95% dimethylpolysiloxane. The column has very low bleed characteristic that provide excellent inertness and can detect methamphetamine with high sensitivity (BGB Analytik, 2025).

Temperature programming is another key optimisation factor in GC-FID. By adjusting the temperature profile of the GC oven, analysts can improve the separation of compounds based on their volatility. In the study by Premathilaka et al. (2020), the temperature programme began at 110°C for the first four minutes, which allowed for the initial equilibration of the sample before ramping up to 280°C. The ramping rate of 10°C per minute had provided a controlled environment to separate methamphetamine and any potential interfering compounds that might co-elute at lower temperatures. Such precise temperature programming ensures that methamphetamine is detected at its optimal retention time, reducing the chances of interference from other substances and improving the overall specificity of the method.

2.3 Carrier gas flow rate and detector settings

The carrier gas, typically helium or nitrogen gas, serves to transport the sample through the column during GC analysis. The flow rate of carrier gas directly affects the efficiency of separation and the time it takes for the compounds to pass through the column. Mitrevski and Zdravkovski (2004) used hydrogen gas as the carrier gas at flow rate of 3.2mL/min while Premathilaka et al. (2024) used nitrogen gas at a flow rate of 1.0 mL/min. This flow rate was chosen to balance the resolution of methamphetamine from other substances in the sample while ensuring that the analysis was completed in a reasonable amount of time. A lower flow rate could increase resolution, while a higher flow rate could reduce resolution. Optimising this flow rate is essential for balancing speed and resolution, particularly in forensic environments where large numbers of samples may need to be analysed.

Another crucial optimisation step involves the settings of the FID. The FID operates by ionising the analytes in a hydrogen flame and detecting the resulting ions. The sensitivity of the FID could be influenced by several factors, including the detector's temperature and the gases used to support the flame. In the study by Premathilaka et al. (2024), the injector and detector temperatures were set to 260°C and 300°C, respectively while Mitrevski and Zdravkovski (2004) set the injector port to 280°C and detector temperature at 300°C. These temperatures are standard for GC-FID and ensure optimal ionisation and signal generation for methamphetamine.

2.4 Method validation and optimisation

The validation of analytical method is a crucial step in ensuring that they meet the requirements in forensic science. Method validation is a series of laboratory tests using the same specimens as the specimens to be examined to prove that the method

can demonstrate performance that meets predetermined requirements (ISO 17025, 2005). In forensic analysis, method validation ensures that results can be consistently reproduced and relied upon in legal contexts. GC-FID is an established technique that requires the optimisation of various factors to maximise its sensitivity, specificity, and reproducibility. The work by Premathilaka et al. (2024) stands out for its comprehensive validation of GC-FID, optimising important parameters, including column selection, temperature programming, carrier gas flow rate, and detector settings. The method's ability to detect low concentrations with high precision and sensitivity makes it ideal for forensic applications, particularly during drug trafficking investigations.

The study conducted by Mitrevski and Zdravkovski (2004) and Premathilaka et al. (2024) showed that analysis time of 6 and 24.1 minutes respectively. Based on these studies, the retention time of the target substances obtained are varied. The difference in retention times could be due to differences in the chromatographic gas conditions used in the analysis of amphetamine and methamphetamine. The factors which could have affected the retention time in gas chromatography analysis included the oven temperature, column type and gas flow in the column (Bizzo et al., 2023). These factors play important role in optimising GC-FID for methamphetamine quantification focusing on method validation aspects, particularly the linearity, precision and accuracy.

2.4.1 Linearity

One of the most critical aspects of validating a method like GC-FID for forensic analysis is establishing the linearity of the method over the concentration range of interest. For the GC-FID method to be reliable in forensic applications, it must exhibit a strong linear relationship between the peak area and methamphetamine concentration.

Calibration curves constructed by injecting known concentrations of methamphetamine standards into the GC-FID system. By plotting the peak area versus concentration, analysts can establish the linearity of the method and determine the concentration of methamphetamine in unknown samples.

Comparing Premathilaka et al. (2024) study with Mitrevski and Zdravkovski (2004) study, Mitrevski and Zdravkovski demonstrated more excellent linearity, as evidenced by the high correlation coefficients of methamphetamine with value of 0.9999. Not only that, the correlation coefficient for other amphetamines also exhibited excellent value which were 0.9999 for amphetamine (AM), 0.9996 for 3,4-methylenedioxyamphetamine (MDA), 0.9998 for 3,4-methylenedioxymethamphetamine (MDMA), 0.9997 for 3,4-methylenedioxy-N-ethylamphetamine (MDEA) and 0.9994 for N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB) while Premathilaka et al. (2024) reported correlation coefficient of methamphetamine of 0.9978 and 0.9985 across concentration ranges of 50-400 mg/L and 10-50 mg/L, respectively.

This suggested that Mitrevski and Zdravkovski (2004) method was highly reliable and could quantify methamphetamine accurately at both high and low concentrations. Such good linearity, is critical for analysing seized tablets that may contain varying amounts of the drug. The linearity of the calibration curve ensures that the method can provide accurate quantification over a broad range of concentrations, making it useful for both routine analysis and high-concentration samples typically encountered in forensic cases.

2.4.2 Limit of Detection (LOD) and Limit of Quantification

LOD and LOQ are two other key validation parameters in determining method's sensitivity. LOD refers to the smallest amount of methamphetamine that can be detected with a high degree of confidence which is useful in cases where the drug is diluted or mixed with other substances, while the LOQ represents the lowest concentration that can be reliably quantified.

Premathilaka et al. (2024) determined the LOD and LOQ of their GC-FID method to be 5.32 mg/L and 5.98 mg/L, respectively. The LOD and LOQ in Rajagopal et al. (2024) study was 0.1454 ppm and 4.4070 ppm respectively. Besides that, Mitrevski and Zdravkovski (2004) study reported LOD and LOQ, 0.7 μg/mL and 1.4 μg/mL respectively. Rajagopal showed more sensitivity in LOD but Mitrevski and Zdravkovski showed more sensitivity in LOQ. Both LOD and LOQ are important for forensic analysis, as methamphetamine in seized tablets can often be found in small quantities. A low LOD and LOQ ensure that even minute traces of the drug can be detected and quantified, making the method highly sensitive for forensic purposes.

While Premathilaka's study calculated LOD and LOQ using mean and standard deviation of the blank signal, a study from Rajagopal et al. (2024) used signal-to-noise (S/N) ratio technique which measured directly from chromatogram. In chromatography, the LOD is the injected amount that results in a peak with a height at least three times higher than the baseline noise level (S/N: 3/1). LOQ then was determined based on the standard concentration of ATS drugs with a peak height of ten times higher than the baseline noise level (S/N: 10/1).

2.4.3 Precision

Precision is an important factor in method validation. It refers to the reproducibility and repeatability of results with different levels of calibrations under identical experimental conditions. In these studies, the precision of the GC-FID method was evaluated by calculating the relative standard deviation (RSD) of replicate injections. Work by Premathilaka et al. (2024) showed their RSD values of three different concentrations were found to be below 2%, indicating that the method was highly precise and could consistently produce reliable results. Mitrevski and Zdravkovski (2004) also interpret their reproducibility results as coefficient of variation (CV). They analyse the test with two different concentrations at 2.4 μg/mL and 7.0 μg/mL and showed good result where the CV percentage were 2.6% and 1.2% respectively.

In determining precision, reproducibility data is better than repeatability because the bias of measured data due to differences in measurement time can be monitored while repeatability cannot be monitored so that it only shows instantaneous data (Haryanto et al., 2011). Precision and accuracy of test data determine the level of laboratory competence, this can be achieved if the quality management system has been implemented effectively and consistently (Ulfiati et al., 2017).

2.4.4 Accuracy

The accuracy of analytical methods is paramount for ensuring the reliability of test results. Accuracy should be established across the reportable range of an analytical procedure and is typically demonstrated through comparison of the measured results with expected values (ICH Q2, 2023).

From the study conducted by Hartono et al. (2023), the determination of amphetamine and methamphetamine in human urine evaluated the recovery rates of

amphetamine and methamphetamine at standard concentrations of 50 μg/L and 100 μg/L, respectively and reported recovery rates was 101.9% for amphetamine and 95.2% for methamphetamine. Recovery of the analyte need not to be 100%, but the extent of recovery should be consistent (for all concentrations tested), precise and reproducible (UNODC, 2009). The recovery rates from Hartono et al., (2023) study showed that the values meet the validation guideline besides demonstrating minimal variability in the results. According to Hortwiz's theory, the recovery of a measurement is influenced by the concentration of the element being measures (Haryanto et al., 2023).

Good accuracy indicates that the method provides results that are unbiased and represent the true concentration or amount of the analyte. It ensures that the method can be trusted to give accurate results. Accurate method provides consistent results across different laboratories which is crucial for standardisation.

2.5 Use of internal standards in GC analysis

The use of internal standards is a common strategy in analytical chemistry to improve the accuracy of quantitative analyses. An internal standard is a compound that is chemically similar to the analyte but not present in the sample. By adding a known amount of internal standard, such as phenylbenzylamine (PBA), to the sample, analysts can correct for any variations in sample injection, chromatographic performance, and detector response. This approach helps to reduce errors and ensures more accurate quantification of the analyte (Moylan et al., 2021).

A study conducted by Mitrevski and Zdravkovski (2004) used diphenylamine as their internal standard in determination of six underivatised amphetamines whereas Premathilaka et al. (2024) used PBA at concentration of 0.8 mg/mL which allowed for the correction of any fluctuations in the FID response and improved the accuracy of

methamphetamine quantification. By utilising internal standards, the instrumental inconsistencies during analysis of samples will not affect the results thus providing more reliable and reproducible data.

2.6 Validation and quantification of methamphetamine in forensic narcotic analysis

The ability to quantify methamphetamine accurately and sensitively is crucial in legal cases, as the amount of drug present in a sample often impacts the severity of charges in drug trafficking and possession cases.

A study conducted by Hartono et al. (2023), validated gas chromatography-mass spectrometry (GC-MS) method for detection of amphetamine and methamphetamine in urine samples. Without doubt, GC coupled to a MS detection system is the most powerful technique for identification and confirmation of amphetamines (Mitrevski and Zdravkovski, 2004). The process of laboratory proof of the drugs can be conducted by examining specimens such as blood, urine, saliva, sweat and hair. Urine is the preferred specimen used for laboratory examination because urine samples are easy to obtain and the concentration of amphetamine or methamphetamine contained in urine is higher than other specimens (Hartono et al., 2023, Alfian and Taufik, 2017).

Hartono et al., (2023) demonstrated that GC-MS method was sensitive to measure amphetamine and methamphetamine in urines where regression coefficient was 0.9972 and 0.9992 respectively. Limit of detection (LOD) and limit of quantification (LOQ) for amphetamine were 2.962 µg/L and 9.873 µg/L respectively while LOD and LOQ for methamphetamine were 6.757 µg/L and 22.253 µg/L respectively. Percentage recovery for amphetamine and methamphetamine were

101.9% and 95.2% respectively while %RSD for reproducibility of amphetamine and methamphetamine are 3.83% and 6.74% respectively.

Premathilaka et al. (2024) developed and validated a GC-FID method specifically for the quantification of methamphetamine in illicit drug samples in Sri Lanka. The study was pivotal, as it addressed the increasing concern over methamphetamine abuse and trafficking in the region. The study analysed forty samples received from the Government Analyst's Department and their purity determined to be ranged from 14% to 96%. Their method was successfully applied to quantify methamphetamine in seized samples and proved to be relatively rapid, simple, precise and suitable for routine forensic analysis.

Rajagopal et al. (2024) performed a study where they quantitated ATS including AM, methamphetamine (MAMP), MBDB, MDA, MDEA, and MDMA in nail samples that collected from male drug abusers in Malaysia. Prior to the analysis, they choose GC-FID method to be validated. R^2 obtained from linearity test showed value of 0.9237, while LOD and LOQ obtained were 0.1454 ppm and 4.4070 ppm respectively for methamphetamine. From the study, the highest content of ATS drug detected in nail samples was MDA analyte which was detected is 83. The second most detected ATS drug was AMP analyte, followed by MDEA, MBDB and MAMP. The least amount of ATS drug detected in nail samples was from MDMA analyte. Out of 88 nail samples, 28 samples was detected having methamphetamine. From the quantification analysis, it showed that the range concentration for methamphetamine contained in nail samples was between 0.3056 to 35.5641 ppm. The results indicated that the proposed methods can be used for ATS drug determination in human nail samples.

2.7 Detection of methamphetamine in forensic narcotic analysis

Identification of amphetamines, especially in forensic cases, is performed by GC-FID as the most rational and universal separation and identification technique. Although GC-FID may lack superior sensitivity and specificity of GC-MS, GC-FID has the advantage of simplicity, reproducibility and a wide range of linearity (Mitrevski et al., 2005). The quantification of methamphetamine in seized drug samples is essential for determining the quantity of the drug, which is often linked to the severity of the charges in legal proceedings.

Direct determination of amphetamines is often followed with absorption in the injector system and column, or their degradation and resulting in peak tailing in the chromatograms or even unsuccessful baseline separation. To avoid these undesirable effects, the polar amino groups are being derivatised to transform them into the corresponding acyl, alkyl and other derivatives of amphetamines. They are more stable and lead to improved chromatographic behaviour including greater sensitivity, better separation and more symmetrical peaks. However, derivatisation also has disadvantages over the direct analysis (Mitrevski et al., 2005). More sample preparation procedure involved, and more time needed besides increasing the possibility of introducing contaminants and producing strong acid byproducts that can significantly alter the column's properties.

Mitrevski and Zdravkovski (2004) had chosen GC-FID as a rapid method for direct simultaneous determination of AM, MAMP, MDA, MDMA, MEA and MBDB in seized tablets without derivatisation. The separation of all six underivatised amphetamines are successful. The authors used a simple procedure for sample preparation and allows separation of amphetamines on SBP-50 capillary column in about 6 minutes. Amphetamines, although analysed directly without derivatisation, they

eluted as very symmetrical peaks. Thus, this method was advantageous over methods where amphetamines must be derivatised prior to separation and detection due to peak tailing. To conclude, the GC-FID method was a simple, fast and precise method that can be used for identification of amphetamines and direct determination in seized tablets, especially in laboratories with heavy workload.

Different from Mitrevski and Zdravkovski (2004) study that used tablet samples, Rajagopal et al. (2024) study choose nail samples. The samples were decontaminated, ATS drug then extracted and the nail samples derivatised prior to the quantification analysis. The purpose of decontamination was to remove external interferences without extracting the analyte of interest from sample matrix. The extracted ATS then derivatised using ethyl acetate and pentafluoropropionic anhydride (PFPA) that act as derivatising agent. The main purpose of derivatising liquid samples before GC analysis is to enhance the chromatographic separation, response of the analyte and peak shape. The presence of ATS drugs including amphetamine, methamphetamine, MDA, MDMA, MDEA and MBDB in the nail samples were detected and identified using GC-FID.

GC-FID is often preferred in routine forensic settings due to its faster analysis times and lower operating costs (Ravindran et al., 2021). Methamphetamine samples seized in the field may not always be pure, and they often contain other substances that could affect the analysis. GC-FID's ability to separate complex mixtures and provide clear chromatographic peaks ensures that methamphetamine can be accurately quantified, even in the presence of other substances (Moylan et al., 2021). This makes GC-FID a versatile and reliable method for forensic analysts working in the law enforcement environments.

2.8 Roles of GC-FID in drug trafficking cases

In drug trafficking cases, the quantity and purity of methamphetamine plays a crucial role in determining the scale of the operation and the severity of the charges. Forensic chemists often rely on GC-FID to provide precise and accurate measurements of methamphetamine, which can then be used as evidence in court. The ability to quantify the drug with a high degree of accuracy ensures that the legal process is fair and that the results are admissible in court.

The use of GC-FID in forensic toxicology also may extends to the investigation of overdose deaths or cases involving drug-facilitated crimes, such as trafficking and distribution. By quantifying the methamphetamine in bodily fluids or other biological samples, forensic toxicologists can help determine whether the drug played a role in a particular incident. In overdose cases, for example, GC-FID can help establish the level of methamphetamine in the victim's system, providing crucial evidence that could support a cause-of-death determination.

Furthermore, GC-FID's ability to detect methamphetamine in a wide range of samples from powders and tablets to liquids and biological matrices makes it valuable for law enforcement agencies. As drug traffickers increasingly rely on sophisticated methods to conceal and transport drugs, the ability to accurately detect and quantify methamphetamine in diverse forms enhances the capacity of forensic chemists to investigate and disrupt illicit drug networks.

2.9 Advantages of GC-FID in forensic laboratories

The primary advantages of GC-FID in forensic chemistry lie in its high sensitivity, accuracy, and reliability. GC-FID is particularly effective for analysing volatile compounds like methamphetamine, which are commonly encountered in

forensic drug analysis. The method is known for its ability to detect low concentrations of analytes, making it ideal for the quantification of methamphetamine in samples that may contain small amounts of the drug.

In forensic laboratories, where large numbers of samples must be processed quickly and quantitate accurately, GC-FID offers significant advantages. While GC-MS provides more detailed information about the molecular structure of compounds, GC-FID has the ability to generate consistent, reproducible results make it a valuable tool for both qualitative and quantitative analysis in forensic applications.

CHAPTER 3

METHODOLOGY

3.1 Instrument

Analysis was performed using a GC coupled with FID (Agilent 7890A). ChemStation software (Rev. B.04.02, Agilent, Santa Clara, CA) was used for GC automation and data analysis. The chromatographic separation was performed using HP-5 MS column, 30 m x 320 μm x 0.25 μm (Agilent J&W Scientific, Folsom, CA, USA). The procedure in preparing methamphetamine samples used sonicator (Sonicor, USA) to mix the particles with methanol.

3.2 Chemicals and solvents

- Pure methamphetamine (99.2% purity) obtained from the Department of Chemistry Malaysia
- Methanol (Merck, EMD Millipore Corporation, Germany)
- Methamphetamine tablets obtained from the Department of Chemistry Malaysia

3.3 Materials and apparatus

- 100 mL and 10 mL volumetric flask
- 5 ml measuring cylinder
- 100 μL micropipette
- 0.45 μm polytetrafluoroethylene (PTFE) syringe filter
- 5 mL Syringe
- 2 mL gas chromatography vial

- Dropper
- Mortar and pestle
- Spatula
- Laboratory scale

3.4 Preparation of methamphetamine stock solution

Pure methamphetamine standard with 99.2% purity was weighed at 100 mg and transferred into 100 mL volumetric flask. Methanol added until reached the graduated mark of the volumetric flask. The volumetric flask was then shake upside down slowly to ensure complete dissolution of the methamphetamine standard. The concentration of the prepared stock solution was 1 mg/mL. It was stored at 5 ± 3 °C when not used.

3.5 Preparation of serial dilution

Serial dilution was performed to prepare with ten different concentrations of methamphetamine calibration standard solutions from 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 µg/mL. 5 mL of stock solution with concentration of 1000 µg/mL was transferred into another 10 mL volumetric flask to produce concentration that was half of the previous concentration. Methanol was added until reaching the graduated mark. The volumetric flask was then shaken upside down slowly to mix the solution. Then, a calibration standard solution carrying concentration of 500 µg/mL was ready. The procedure was repeated until a solution of concentration with 1.95 µg/mL prepared. 1 mL of each concentration was inserted into GC vial and labelled before analysed. All solutions were then stored at 5 ± 3 °C when not used.

3.6 Preparation of quality control standards

Three quality control standard solutions were prepared with concentration of 10, 50 and 300 μg/mL. Firstly, to prepare a solution with 10 μg/mL, 0.1 mL of methamphetamine stock solution inserted into 10 ml volumetric flask and labelled as quality control 1 (QC 1). Next, 0.5 mL of methamphetamine stock solution added into new 10 ml volumetric flask and labelled as quality control 2 (QC 2) to produce concentration of 50 μg/mL. Lastly, for quality control 3 (QC 3), 3 ml of methamphetamine stock solution inserted into new 10 ml volumetric flask for a concentration of 300 μg/mL. Then, methanol was added into all volumetric flask until reaching the graduated mark. The volumetric flask was then shaken up and down slowly to mix the solution. 1 mL of each quality control standard solutions were then inserted into GC vial and labelled. The volume of methamphetamine stock solution needed to produce solutions with concentrations of 10, 50 and 300 μg/mL were calculated using Equation 1 as follows:

$$M_1V_1 = M_2V_2 \qquad \text{Equation 1}$$

where, M_1 is the initial concentration of stock solution, V1 is the initial volume needed from stock solution, M_2 is the final concentration of diluted solution and V_2 is the final volume of diluted solution.

3.7 Preparation of methamphetamine samples

In this study, a total of 155 methamphetamine tablets samples crushed into powder using mortar and pestle. 4 ± 0.3 g of methamphetamine powders accurately weighed and inserted into GC vial. Then, 1 mL of methanol added into the vial and sonicated for 5 minutes. The solution was then filtered using 0.45 μm

polytetrafluoroethylene (PTFE) syringe filter. All samples were properly labelled from P001 until P155.

3.8 Operating conditions for GC-FID

A GC-FID was used for the method validation and analysis of methamphetamine tablet samples. Table 1 shows the operating parameters of the GC method, adapted from Quah et al., (2023).

Table 3.1 Operating parameter for methamphetamine analysis using GC-FID

Parameter		**Condition**
Column		HP-5 MS 30 m x 320 μm x 0.25 μm
Injection mode		Splitless
Injector temperature		300 °C
Oven temperature ramp program	Carrier gas	Purified nitrogen gas
	Initial temperature	70 °C
	Start time	2 min
	Temperature rate	20 °C/ min
	Final temperature	280 °C
	Final time	1 min
Detector	Temperature	325 °C
	Gas	Hydrogen gas at 30 mL/min
Analysis time		14.5 min