

**SIMULTANEOUS DETERMINATION OF  
METHANOL AND ETHANOL IN LOCAL AND  
FOREIGN ALCOHOLIC DRINKS BY GC-FID**

**MANJA MURNI BINTI CHE KALID**

**UNIVERSITI SAINS MALAYSIA**

**2022**

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LOCAL AND FOREIGN ALCOHOLIC DRINKS BY GC-FID**

**by**

**MANJA MURNI BINTI CHE KALID**

**Thesis submitted in partial fulfilment of the requirements  
for the degree of  
Master of Science (Forensic Science)**

**September 2022**

## ACKNOWLEDGEMENTS

Praise the All Mighty, I successfully completed my thesis on time. First and foremost, I would like to express my heartfelt gratitude to my supervisor, Associate Professor Dr. Nik Fakhuruddin bin Nik Hassan, for his constant wisdom throughout my studies. It's a lesson I'll keep in mind for the rest of my life. I would also like to thank the Faculty of Health Science, Universiti Sains Malaysia, particularly Dr. Nurasmah binti Mohd Shukri, for their contributions, as well as my colleagues at KIMIA Pahang, especially Dr. Faridah binti Mohd Marsin, for their guidance when needed.

I am also grateful to the Malaysia Department of Chemistry (Kimia Malaysia) for providing me with the opportunity to further my education. A special thanks goes out to the staff at the forensic toxicology laboratory at KIMIA Malaysia for giving me the time and space to use scientific tools throughout my laboratory work. I would also like to thank the Public Service Department for approving me to continue my studies through the CBBPTB programme. Tuition fees are also acknowledged as part of the MOU programme between Universiti Sains Malaysia (USM) and KIMIA Malaysia.

My deepest appreciation goes to my family, particularly my wonderful mother, Wan Jah binti Wan Salleh, who has been my entire life, supported, inspired, and contributed so much to the making of this dissertation. My siblings, my aunt Wan Hasmah binti Wan Salleh, who never fails to support me in every way, and my friends, who put up with me through thick and thin. My final dedication is to my late father, Che Khalid bin Ismail, who has been my driving force to continue moving forward.

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## LIST OF SYMBOLS

°C	Degree Celsius
%	Percent
°C/min	Degree Celsius per minute
μL	Microliter
μm	Micrometer
As	Peak asymmetric factor
C	Peak area
g	Gram
g/cm <sup>3</sup>	Gram per cubic centimeter
g/mol	Gram per mol
k	Retention factor
L/kg	Liter per kilogram
m	Meter
mg/dL	Milligram per deciliter
mg/L	Milligram per liter
MHz	Megahertz
min	Minute
mL	Milliliter
mL/min	Milliliter per minute
mm	Millimeter
mΩ/cm	Megaohm per centimeter
N	Column efficiency
n	Total number
ng/mL	Nanogram per milliliter

$r^2$	Correlation of coefficient
$R_f$	Resolution
rpm	Rate per minute
$R_t$	Retention time
v/v	Volume per volume
w/v	Weight per volume

## LIST OF ABBREVIATIONS

ABV	Alcohol by volume
ADH	Alcohol dehydrogenase
ADP	Adenosine di-phosphate
ATP	Adenosine tri-phosphate
CNS	Central nerve system
CV	Coefficient of variance
DART-MS	Direct analysis in real time mass spectrometry
EU	European union
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
GC-FID	Gas chromatography flame ionization detector
GC-FTIR	Gas chromatography-Fourier transform infrared spectroscopy
GC-MS	Gas chromatography mass spectrometry
GC-TCD	Gas chromatography thermal conductivity detector
H	High
HS-GC-FID	Headspace gas chromatography with flame ionisation detection
IS	Internal standard
IUPAC	International Union of Pure and Applied Chemistry
L	Lower
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
M	Medium
MRL	Maximum residue limit

MTC	Maximum tolerable concentration
NCADD	National Council on Alcoholism and Drug Dependence
NIR	Near infrared
NMR	Nuclear magnetic resonance
PME	Pectin methyl esterase
PTFE	Polytetrafluoroethylene
QC	Quality control
RM	Ringgit Malaysia
RSD	Relative standard deviation
S/N	Signal to noise ratio
SPE	Solid phase extraction
SST	System suitability test
TA	Total acidity
UNODC	United Nations Office on Drugs and Crime
UV-VIS	Ultraviolet-visible
WHO	World Health Organization

# **SIMULTANEOUS DETERMINATION OF METHANOL AND ETHANOL IN LOCAL AND FOREIGN ALCOHOLIC DRINKS BY GC-FID**

## **ABSTRAK**

Keracunan metanol akibat daripada pengambilan minuman beralkohol adalah isu di seluruh dunia yang semakin berkembang. Metanol boleh menyebabkan masalah kesihatan yang teruk dan membawa kepada kematian. Atas sebab-sebab ini, banyak kaedah untuk mengukur kepekatan metanol dalam minuman beralkohol telah dibangunkan. Matlamat kajian ini adalah untuk membangunkan kaedah yang tepat, sensitif dan mudah dengan menggunakan GC-FID untuk penentuan serentak metanol dan etanol dalam minuman beralkohol jenama tempatan dan asing. Kaedah ini dibangunkan dengan pengoptimuman parameter GC. Parameter yang dioptimumkan termasuk suhu ketuhar awal 40°C yang ditahan selama 2 minit, kenaikan pada 25°C/min sehingga suhu ketuhar 100°C dicapai, kadar aliran gas pembawa 4.5 mL/min, nisbah pisah 2:1 dengan isipadu suntikan 10 µL. Kaedah yang dioptimumkan telah disahkan mengikut garis panduan UNODC dari segi spesifisiti, lineariti, had pengesanan dan pengukuran, kejituan dan ketepatan. Kedua-dua keluk penentuan metanol dan etanol menawarkan kelinearan yang baik, dengan  $r^2 > 0.99$  sepanjang julat 0.001-0.625% untuk metanol dan 5-25% untuk etanol. LOQ masing-masing untuk metanol dan etanol ialah 1.72 mg/L dan 1.76 mg/L dengan ketepatan dan pemulihan yang baik. Kaedah ini memerlukan 5.40 minit untuk menghasilkan keputusan. Kaedah yang telah ditetapkan ini kemudiannya digunakan dengan menganalisis 19 minuman beralkohol yang berbeza dengan menyuntiknya terus ke dalam GC-FID selepas asetonitril ditambah sebagai piawai dalaman. Kajian ini mendedahkan bahawa 36.8% daripada sampel yang diperiksa mengandungi kepekatan metanol antara 0-0.0198%,

manakala etanol dikesan dalam setiap sampel. Kaedah yang dicadangkan adalah sensitif, mudah, dan tidak memerlukan sebarang pra-rawatan, menjadikannya sesuai untuk analisis sampel rutin forensik toksikologi. Yang penting, jumlah surih metanol dapat dikesan dengan pasti tanpa gangguan daripada kepekatan etanol yang jauh lebih tinggi. Kesimpulannya, penyelidikan ini telah menunjukkan bahawa kapilari lajur DB ALC 1 dalam GC-FID dengan parameter yang dioptimumkan adalah sangat selektif dan sangat sensitif dalam pengesanan dan pengukuran serentak metanol dan etanol dalam minuman beralkohol.

# **SIMULTANEOUS DETERMINATION OF METHANOL AND ETHANOL IN LOCAL AND FOREIGN ALCOHOLIC DRINKS BY GC-FID**

## **ABSTRACT**

Intoxication with methanol resulting from the consumption of alcoholic beverages is a worldwide issue that is growing. Methanol can cause severe health issues and lead to fatality. Due to these reasons, numerous methods for measuring methanol concentrations in alcoholic beverages had been developed. The aim of this study was to develop an accurate, sensitive and simple method by using GC-FID for simultaneous determination of methanol and ethanol in local and foreign brands of alcoholic beverages. The method was developed by optimisation of the GC parameters. Optimised parameters included an initial oven temperature of 40°C held for 2 minutes, ramping at 25 °C/min until an oven temperature of 100°C was reached, a carrier gas flow rate of 4.5 mL/min, a split ratio of 2:1 with injection volume of 10 µL. The optimised method was validated in accordance with UNODC guidelines in terms of specificity, linearity, detection and quantification limits, precision and accuracy. Both methanol and ethanol calibration curves offered good linearity, with  $r^2 > 0.99$  throughout ranges of 0.001-0.625% for methanol and 5-25% for ethanol. The respective LOQs for methanol and ethanol were 1.72 mg/L and 1.76 mg/L with good precision and recoveries. This method required 5.40 minutes to yield results. This established method was then applied by analysing 19 different alcoholic beverages by injecting them directly into the GC-FID after acetonitrile was added as an internal standard. This study revealed that 36.8% of the samples examined contained methanol concentrations ranging from 0-0.0198%, whereas ethanol was present in every sample. The proposed method was sensitive, straightforward, and did not require any pre-



treatment, making it suitable for routine toxicological forensic sample analysis. Importantly, trace amounts of methanol were reliably detected without interference from significantly higher concentrations of ethanol. In conclusion, this research had demonstrated that the DB ALC 1 column capillary in GC-FID with optimised parameters is very selective and highly sensitive in the simultaneous detection and measurement of methanol and ethanol in alcoholic beverages.

# CHAPTER 1

## INTRODUCTION

### 1.1 Background of Study

Malaysia is a Muslim country that follows Islamic law. As a result, most Malay eateries do not serve alcohol. Non-Muslims, on the other hand, can still buy alcoholic drinks at hotels, pubs, and restaurants with a western style. These sales contribute to Malaysia's high rate of alcohol use. According to Code Blue, alcohol consumption in Malaysia is becoming more severe and has gradually increased over time (CodeBlue, 2020). In May 2011, the World Health Organization (WHO) ranked Malaysia as the world's tenth biggest consumer of alcohol, even though it has a small population, is small in size, and has a large Muslim population (The Star, 2011).

According to Statista, which was last updated in May 2021, beer is the most popular alcoholic drink in Malaysia. Beer, in fact, is taxed far higher than other alcoholic beverages, with the world's second highest excise duty after Norway (World, 2017). If the excise duty is increased further, the price of the product will rise, which will encourage the illegal market. Hard-core drinkers who can't afford beer will drink traditional or locally made alcoholic drinks or alcohol that was made illegally because it is cheaper.

The primary concern with these illicit alcoholic beverages is whether they are homemade or produced by an unregistered distillery. Typically, they either produce new toxic alcohol or refill and repackage popular brands with cheaper alternatives or a mixture of water, alcohol, and unidentified compounds.

One of the unrecorded compounds added to those brews is methanol, a type of alcohol that is sweeter than the usual ethanol found in alcoholic beverages but it is more harmful. Methanol is actually naturally found in very small amounts in fruit juices products. Pectin consists of methoxy poly-galacturonic acids and is believed to be the source of methanol in plant-based products (Oh, 2020). Methanol is also formed simultaneously with ethanol during improper fermentation and distillation processes (Sirhan *et al.*, 2019). Therefore, most fermented alcoholic beverages contain methanol in addition to ethanol.

Excess methanol in alcoholic beverages can cause poisoning and seriously affect human health, such as blindness or death. Health problems associated with mass poisoning have been observed in recent years. Also, in Malaysia in early 2013, 27 deaths out of 41 victims have been documented as the first outbreak of methanol poisoning in Malaysia (Rahimi *et al.*, 2021). When methanol poisoning reoccurs in Malaysia in September 2018, it will be crucial to have a method for simultaneously detection of methanol and ethanol in alcoholic beverages. In this study, gas chromatography technique was used to develop the simultaneous quantitative determination of methanol and ethanol in imported brands as well as locally produced alcoholic beverages in Malaysia.

## 1.2 Problem Statement

Toxic alcohol poisoning, such as methanol poisoning, is a global problem that is getting worse. Consumption of non-commercial alcoholic beverages is the most common cause of methanol poisoning. This kind of poisoning can also be caused by ingesting methanol itself or drinking alcoholic beverages containing high levels of methanol. Methanol poisoning is a rare occurrence, but it is a very severe poisoning. According to the WHO, methanol poisoning outbreaks have been documented in a number of countries, including India, Norway, Turkey, Australia, and Iran. In Malaysia, the issue of methanol poisoning was raised in 2018, when a total of 64 people had died after drinking cheap alcohol bought from the black market (Md Noor *et al.*, 2020; Rahimi *et al.*, 2021).

Alcoholic beverages are frequently contaminated with methanol. Ethanol and methanol are both colourless liquids with similar tastes, smells, and appearances that are difficult to distinguish by visual inspection. They have similar physicochemical properties and are present as by-products after distillation (Kofi Tulashie *et al.*, 2017). Methanol may be produced in alcoholic beverages as a result of improper distillation or fermentation, or it may be intentionally added to boost alcohol level. Methanol can originate naturally from the degradation of pectins during fermentation (Pineau *et al.*, 2021a).

Illegal production of alcoholic beverages for commercial gain is a severe problem in various countries around the world. There is an issue when non-agricultural ethanol or methanol is mixed into these low-cost drinks to increase the amount of

alcohol while lowering the prices. Many cases were reported pertaining to methanol poisoning when the less expensive and easy-to-obtain methanol was substituted for ethanol in alcoholic beverages (Vaskova, 2014). This method of counterfeiting alcoholic drinks was also the cause of widespread methanol poisoning in Malaysia.

The counterfeit or adulterated alcoholic drinks contain much higher amounts of methanol, which can cause headaches, dizziness, and even blindness among other symptoms, and of course, death as well. Methanol becomes hazardous in the body when its metabolites to formaldehyde and formic acid. Methanol has maximum residual limits (MRL) and maximum tolerable concentrations (MTC), and exceeding these limits can result in toxicity. The legal limits that were established by the EU of methanol in alcoholic beverages are 0.4% (v/v) (4000 mg/L) (Paine and Dayan, 2001a). The ingestion of as little as 10 mL of methanol can cause permanent blindness and 30 mL can be fatal (Kofi Tulashie *et al.*, 2017).

Recently, a large number of potentially lethal alcoholic beverages containing methanol in concentrations exceeding legal limits were unlawfully distributed as well as sporadic cases (Arslan *et al.*, 2015). This activity will result in a greater number of people being poisoned and dying. Because of this issue, this study focused on the detection of methanol and ethanol concentrations in both local and foreign alcoholic beverages, as not all dangerous beverages have been evaluated in previous studies, particularly in Malaysia. This study will lead to the measurement of alcohol concentration in alcoholic beverage samples, which can be utilised to prevent indirectly unlawful methanol manufacturing.

### **1.3 Aims and Objectives**

The aim of this research was to develop a simple, sensitive, and direct method for determination of methanol and ethanol in local and foreign alcoholic beverages using a gas chromatography system coupled with a flame ionisation detector.

The specific objectives of this study were:

1. To develop and validate a gas chromatographic method for determination of methanol and ethanol simultaneously.
2. To apply the established method for determination of the methanol and ethanol concentrations in local and foreign alcoholic beverage samples.

### **1.4 Significance of the Study**

As the number of cases of methanol poisoning from alcoholic beverages rises, there is a growing demand for good sensitive and faster methods of simultaneously measuring the concentrations of methanol and ethanol in alcoholic beverages. The new method is anticipated to be applicable for routine analysis at the Chemistry Department of Malaysia in place of the current method (Kimia Malaysia). It is also expected that the results of this study will be used to support clinical cases of deaths caused by methanol. Additionally, this study could initiate further research on methanol levels detection in local beverages in order to set a maximum residue limit in Malaysia's food regulations, which would subsequently affect the licensing of alcohol manufacturing.

## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **2.1 Overview**

This chapter provides a compilation of previous studies on the methanol and ethanol concentrations in various types of alcoholic beverages, as well as their most recent detection and quantification techniques. This chapter also focuses on the efficiency and sensitivity of a method for the preparation, separation, and simultaneous determination of methanol and ethanol.

#### **2.2 Methanol and Ethanol**

The word "alcohol" comes from the Arabic word al-kuhul, which means "kohl" (Hamdan- Mansour, 2016). This word has nothing to do with liquids or intoxicating substances. However, the term "kohl" originally referred to a fine black powder similar to eye shadow that was used to colour the eyes darkly. Tapsoba and his colleagues reported that kohl was mostly made up of four chemicals: galena, cerussite, laurionite, and phosgenite (Tapsoba *et al.*, 2010). Kohl was made by a process called sublimation, in which a solid is turned into a vapor and then back into a solid to make a very fine black powder. Around the 16th century, the term was adopted into English to describe any fine powder (Erict, 2016). Later, the word "alcohol" was used to describe anything made through distillation and sublimation, including fluids. And now, the word "alcohol" just means spirit-based ethanol or alcoholic drinks.