ANALYSIS OF GAHARU ESSENTIAL OIL USING GC/GCMS

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ANALYSIS OF GAHARU ESSENTIAL OIL USING GC/GCMS

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A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
University College of Engineering & Technology Malaysia

November, 2006

DECLARATION

I declare that this thesis entitled “Analysis of Gaharu Essential oil Using GC/GCMS.” is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.”

Signature :…….

Name of Candidate : Shahrizal Bin Ramli

Date : November 20th , 2006

To my beloved mother, father, brother, sister and friends..

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ABSTRACT

In this study, 7 samples of Gaharu essential oil from the industries was analyzed using Gas Chromatography/ Gas Chromatography Mass Spectrometry (GC/GCMS). All the 7 samples (G1, G2, G3, SP001, SP002, SP003 and SP004) was identified and analyzed to determine the best quality of Gaharu essential oil. Difference grade of Gaharu contain different percent and different compounds in the essential oil. The best quality of Gaharu essential oil is depends on the important chemical components that contribute to the special characteristic of aroma to Gaharu and the number of components exists in all samples. Agarospirol, jingkoh eremol and kusenol are the important compounds that contribute the characteristic of aroma to Gaharu. Every single oil normally has more than a hundred components and it can be detected through analyzing the essential oil with a chromatograph. The samples will be analyzed by GC/MS equipment. GC/MS equipment have been choose to analyze the chemical components in Gaharu essential oil because this equipment make an effective combination for chemical analysis. The result of this study, sample Gaharu essential oil, G1 is the best quality of essential oil recommended by industry in Malaysia.

ABSTRAK

Dalam kajian ini, beberapa contoh minyak Gaharu yang telah diambil daripada ondustri akan dianalisa dengan mengunakan alatan Gas Chromatography/ Gas Chromatography Mass Spectrometry (GC/GCMS). Terdapat 7 sampel minyak Gaharu (G1, G2, G3, SP001, SP002, SP003, dan SP004) yang dikenalpasti dan dianalisa untuk menentukan contoh minyak yang paling berkualiti. Setiap kayu Gaharu mempunyai kualiti yang berbeza dan ianya akan menghasilkan minyak Gaharu yang berbeza dari segi kehadiran komponen terpenting dan jumlah komponen yang hadir dalam setiap contoh minyak. Minyak Gaharu yang berkualiti ditentukan oleh kehadiran komponen terpenting yang memberikan sifat istimewa pada minyak Gaharu dari segi aromanya. Komponen terpenting ini ialah agarospirol, jinkoh eremol dan khusenol. Minyak Gaharu boleh terdiri daripada lebih seratus komposisi kimia yang dapat ditentukan menggunakan GC/GCMS. Alatan ini dipilih kerana ianya dapat mengesan kehadiran setiap komponen dengan baik. Selepas analisa dijalankan, contoh minyak G1 merupakan contoh minyak Gaharu yang paling berkualiti berdasarkan pengalaman dan rujukan daripada pihak industri di Malaysia.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

GC	Gas Chromatography -
MS -	Mass Spectrometry
GC/MS	Gas Chromatography- Mass Spectrometry
P -	Pressure
T	Temperature
F	Flowrate
Aquilaria	One if the Gaharu species
Agarwood	Others name of Gaharu
RI	Retention Time
KI	Kovet Index

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.0 Introduction

Gaharu is one of the most expensive woods in the world. It is the occasional product of two to four genera in the family Thymelaeaceae, with *Aquilaria agallocha* and *Aquilaria malaccensis* the best known species. *Aquilaria malaccensis* is a large evergreen tree reaching a height of 25 m or more **[1].** Gaharu oil is a mixture of sesquiterpenes, sesquiterpene alcohols, chromone derivative and resin [**2**].

Gaharu essential oil may consists up to 100 chemical components that give a special characteristic to gaharu essential oil. Agarospirol, jingkoh eremol and kusenol are the important compounds that contribute the characteristic of aroma gaharu [**4**]. Every single oil normally has more than a hundred components and it can be detected through analyzing the essential oil with a chromatograph [**5**].

7 samples of Gaharu from industry (G1, G2, G3,SP001, SP002, SP003 and SP004) will be analyze by GC/MS to identify the best quality of gaharu essential oil. This type of analysis gives information about the individual components of each oil, and their relative amounts. GC/MS equipment have been choose to analyze the chemical components in gaharu essential oil because this equipment make an effective combination for chemical analysis [**3**]. GC/MS is a combination of Gas Chromatography (GC) equipment and Mass Spectrometry (MS) equipment. GC analysis common conformation test. This equipment will separate all components in

a sample and provides a represent spectral output. MS equipment meanwhile commonly used in arson investigation, petroleum product analysis and so on

Common name for Agarwood [6]:-

- Agar wood
- Jin Koh
- Aloes wood
- Gaharu
- Eagle Wood
- Jinkoh
- Oud

Species Of Agarwood [**7**]:-

- Aquilaria agallocha
- Aquilaria grandiflora
- Aquilaria ophispermum
- Aquilaria sinesis
- Aquilaria crassna
- Aquilari malaccensis
- Aquialaria pentandra
- Aquilaria yunnanensis

Table 1.0: Scientific Classification of Gaharu [**7**]

Scientific Classification	
Kingdom	Plantea
Division	Mangnoliophyta
Class	Mangnoliopsida
Order	Malvales
Family	Thymelaeaceae
Genus	aquilaria

1.1 Objective of the study

The objective of this study is: -

1) To developed a standard of industrial grade of Gaharu essential oil quality based on the chemical constituent.

1.2 Scope of this study

In this study, 7 samples of Gaharu essential oil (G1, G2, G3, SP001, SP002, SP003, and SP004). will be analyze using GC/MS from industry was analyzed using GC/GCMS.

1.3 Problem statement

In Gaharu industrial, actually there are no standard that can be refered to identified and to determined which is the best quality of Gaharu essential oil Nowadays, the best quality of gaharu essential oil is determined by trander and customer and not based on the chemical components exist on the product. This is happen because there is no standard to determined the best quality of Gaharu essential oil. Gaharu essential oil may consist up to 20 components and it can de analyzed by GCMS.

CHAPTER 2

LITERATURE REVIEW

2.0 Essential oil

Each of the essential oils used in aromatherapy can be used either alone or in combinations to create a desired effect. Essential oil is also known as volatile oil and ethereal oil [**9**]. The essential oil are found in different parts of the plant such as the flowers, twigs, leaves and bark, or in the rind of fruit. For example, in roses essential oils are found in the flowers, in basil it is in the leaves, in sandalwood in the wood, and so on.

According to Ghannadi, the essential oil was analyzed by GC/MS **[9].** Pure essential oils are expensive, but they are also highly effective. Only a few drops at a time are required to achieve the desired aromatherapy effects.

Synthetic oils are available at a lesser price, but they do not have the healing aromatherapy powers of the natural oils. Essential oils have an immediate impact on our sense of smell, also known as olfaction when an essential oil is inhaled, olfactory receptor cells are stimulated and the impulse is transmitted to the emotional center of the brain, or limbic system.

2.1 Uses of Essential Oil

In Malaysia, there are recorded of the use of the gaharu in various folk remedies for the treatment of weakness, stomach pains, in pregnancy, after delivery, fever, chest pains, body pains, rheumatism, women dieses and dropsy[**11].** It is reputed to be somatic and sedative, has antibiotic, anti-tumor and anti cancer effect **[12]**

2.1.0 Vaporization

The essential oils can help freshen a room or be used to create a special mood. The oils can be used in an oil burner, on a light bulb ring, or in a room spray

2.2 Aromatherapy

Aromatherapy is an art, and science, of using essential oil extraction from aromatic response. Essential oil can be combined with massage oil or bath salt to enhance health and beauty.

The essential oil taken from plants and used in Aromatherapy have been describe as their “life force”- they are essential oil the plants biological process, as well as being the substance which gives them their scent. The limbic system is connected to areas of the brain linked to memory, breathing, and blood circulation, as well as the endocrine glands which regulate hormone levels in the body. The properties of the oil, the

fragrance and its effects, determine stimulation of these systems. When used in massage, essential oils are not only inhaled, but absorbed through the skin as well.

They penetrate the tissues and find their way into the bloodstream where they are transported to the organs and systems of the body. Essential oils have differing rates of absorption, generally between 20 minutes and 2 hours, so it is probably best not to bathe or shower directly following a massage to ensure maximum effectiveness. [**10**].

2.3 Aquilaria

Aquilaria are recored for peninsular Malaysia and all are believed to be able to produced oleoresins **[13].** A member of the family Thymelaeaceae, *Aquilaria* is a relatively slow-growing, medium-sized tree, on average 15–25 m tall; some of the more than 15 species (e.g., *A. microcarpa*) reach heights of as much as 40 m[**9**]. Having a moderately straight stem, it can achieve a diameter of up to 250 cm, although some species remain considerably smaller and more shrublike, e.g., *A. khasiana.* Most *Aquilaria* species have smooth, thin, pale gray bark with dense, dark foliage of shiny elliptical to oblong leaves (7.5–12 cm long by 2.5–5.5 cm wide).

Aquilaria regenerates freely under natural conditions as seedlings around the mother tree or sprouts from the stumps of harvested trees. However, mother trees are becoming scarce in many areas because of over-exploitation. Although this condition may not lead to local extinction of the species, it may severely affect the availability of the product and, thus, the local *gaharu* economy.

2.4 Agarwood

Agarwood or eaglewood is one of the most expensive woods in the world. It is the occasional product of two to four genera in the family *Thymelaeaceae*, with *Aquilaria agallocha* and *Aquilaria malaccensis* the best known species [7].

Agarwood is known as “jinko” in Japan, which translates as “sinking incence” or “ incence that sinks in water, “ due to the weight of the resin in the wood.Agarwood, belongs to the genus Aquilaria and to the species Agallocha. It is native to Cambodia, Laos, Vietnam, Indonesia and Northern India, although resources in many of these areas have suffered from unchecked exploitation in recent times. Agarwood tree is a large evergreen tree reaching a height of 25 m or more **[1]**.

The tree bears a fragrant, green and yellowish-white flower (shown on the right Aloeswood is not related to Aloe Vera (Latin name: Aloe barbadensis). Agarwood has a deep, woodsy scent, often described as warm and earthy. The aroma is distinctive and very penetrating. Because the scent is so pleasant, agarwood is used to make essential oil and aloeswood chips, and it is also a prized ingredient of incense in the Middle East and in Japan. The fragrance of aloeswood can vary greatly depending on the country of origin, the density of resin and depending also on the part of the tree from which it is harvested.

2.4.1 Formation of The Gaharu Oil

Formation of agarwood occurs in the trunk and roots of trees that have been infected by a fungus. As a response, the tree produces a resin high in volatile organic compounds that aids in suppressing or retarding fungal growth.

While the unaffected wood of the tree is relatively light in color, the resin dramatically increases the mass and density of the affected wood, changing its color from a pale beige to dark brown or black .The effect of the dead tree's immune response on this particular fungus produces chemical components that bond to multiple receptor sites in the human olfactory organ, as does the scent of truffle in that of swine, producing a hypnotic, endlessly complex perfume useful in most perfumery compositions even in minute traces.

Figure 2.0 : Alosewood tree in South Kalimantan

Source: David Oller and Kyozaburo Nakata " Chemical Analysis on Aloeswood

Figure 2.1: Young Aloes Wood trees in South Kalimantan

Source : David Oller and Kyozaburo Nakata " Chemical Analysis on Aloeswood

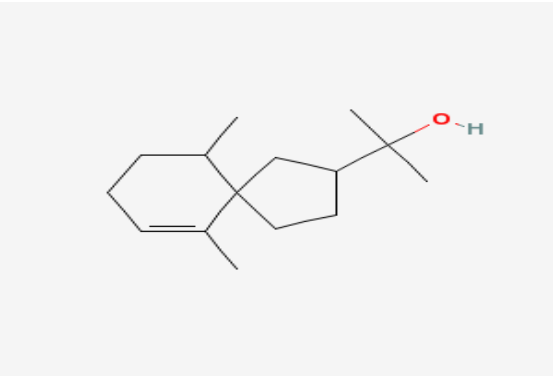
2.4.2 Different Grades of Aloeswood

There are many different grades of aloeswood, which are determined based on the density of resin and the type and intensity of the aroma it produces [**7**]. The lower grades are often used in essential oil, while the higher grades are mainly used for incense. The highest grade of aloeswood is called *Kyara*, which means "precious" in ancient Japanese, and it is more expensive per ounce than gold.

2.5 Agarospirol

Agarospirol is a one of important compounds that contribute the special aroma for gaharu essential oil. The IUPAC name for Agarospirol is 2-(6,10-dimethyl-2-spiro[4.5]dec-9-enyl)propan-2-ol. The formula molecule for Agarospirol is $C_{15}H_{26}O$. This compound has a molecular weight 222.366 g/mol. The functional groups in agarospirol are hydroxyl and alkenes. The structure of Agarospirol is aromatic.

Figure 2.2: The structure of agarospirol

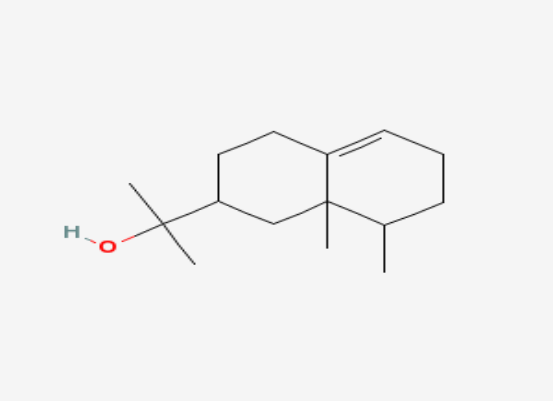


Source: http://www.pubchem.com/agarospirol

2.6 Jinkoh-eremol.

Jinkoh-eremol is a one of important compounds that contribute the special aroma for gaharu essential oil. The IUPAC name for jinkoh-eremol is 2-(8,8a-dimethyl-2, 3,4,6,7,8-hexahydro-1H-naphthalen-2-yl)propan-2-ol. The formula molecule for Jinkoh-eremol is $C_{15}H_{26}O$. This compound has a molecular weight 222.366 g/mol. The functional groups in Jinkoh-eremol is hydroxyl and alkene. The structure of Jinkoh-eremol is aromatic.

Figure 2.3: The structure of Jinkoh-eremol

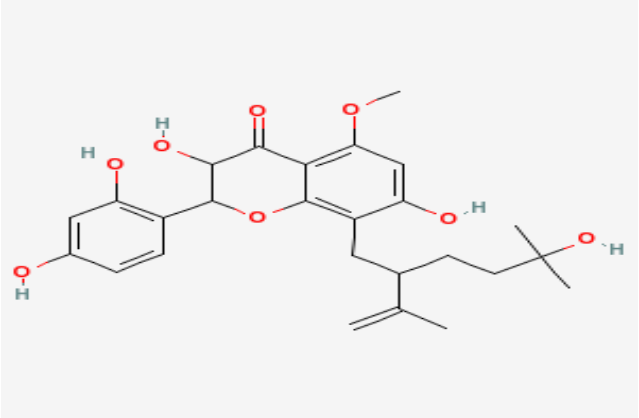


Source : http://www.pubchem.com/jinkoheremol

2.7 Khusenol

Khusenol is a one of important compounds that contribute the special aroma for gaharu essential oil. The IUPAC name for Khusenol is 2-(2,4-dihydroxyphenyl)-3,7-dihydroxy-8-(5-hydroxy-5-methyl-2-prop-1-en-2-yl-hexyl)-5-methoxy-chroman-4-one. The formula molecule for Khusenol is $C_{26}H_{32}O_8$. This compound has a molecular weight 472.527 g/mol. The functional groups in Jinkoh-eremol are hydroxyl, alkene, ether, and ester. The structure of Khusenol is aromatic.

Figure 2.4: The structure of Khusenol



Source: http://www.pubchem.com/kusenol

2.8 Gas Chromatography (GC)

There are four different GC introduction techniques and a direct Mass spectrometry technique which each technique have its own advantages and disadvantages **[16].** The function of GC is to separate all chemical components in a sample and provide a representative spectral output. The chemicals sample are injects into injection port of the GC device. The sample will vaporized and then separates and analyzes the various components.

Each chemical components will produces a specific spectral peak and it will be record on electronically or paper chart. Retention time (RI) is duration is needed between injection and elution. The retention time can help to differentiate between some compounds. The size of the peak is proportional to the quantity of the corresponding substances in the specimen analyzed. The peak is measured from the baseline to the tip of the peak. The different duration time for each component take to travel to the top depend on the characteristics of each components [**3**]. The lighter component travel more quickly and some components take longer time due to their shape. The different components interact with each other may hinder or acceleration the component's travel as the components strike each other. The surface characteristics of the components may be important.

GC analysis depends on similar phenomena to separate chemical substances. A mixture of chemicals presents in a specimen can be separated in the GC column. Some chemicals and physical characteristics of the molecules cause them to travel through the column at different speeds. If the molecules has low mass it may travel more swiftly. Also, the molecule's shape also may cause the time needed to exit the column. How the different substances relate to each other may cause the time needed to travel the column to increase or decrease.

Interactions between the sample's molecule and the column surface may molecules that interact with the column differently. Base on journal [**2**], GC analysis of gaharu essential oil from solvent extraction and hydrodistillation were performed with Shimadzu GC 12A capillary chromatography and Shidmatzu GC 2010. A Hewlett-packard HP5890 series II GC equipment with an S.I.S model 971 micro Cryotrap using liquid $C0_{2,}$ and HP 5989A MS Engine were used for analysis [**9**].

2.8.1 Description of GC Process

The equipment used for Gas Chromatography (GC) generally consists of an injection port at one of a metal column packed with substance material and a detector at the other end of the column. A carrier gas propels the sample down the column. Flow meters and pressure gauges is used to maintain a constant gas flow. Carrier gases are usually argon, helium, nitrogen, or hydrogen. The gas that does not react with the sample or column is essential for reliable results.

To ensure proper separation, the sample must enter the column in a discreet, compact packet. Normally the sample is injected into the injection port with a hypodermic needle and syringe or capable of measuring the specimen amount. The needle is stuck into a replaceable neoprene or silicon rubber septum that covers the injection port. The injection port is maintain at a temperature at which the sample vaporizes immediately.

The column is a metal tube, often packed with a sand-like material to promote maximum separation. Columns are commonly obtained pre-packed by vendor. As the sample moves thought the column, the different molecular characteristics determine how

Each substance in the sample interacts with the column surface and packing. The column allows the various substances to partition themselves.

Substance that do not like to stick to the column or packing move through the column rapidly. Substances that do not like to stick the column or packing are impeded but eventually elute from the column. Ideally, the various components in the sample separate before eluting from the column end.

The GC instrument uses a detector to measure the different as they emerge from the column. Among the available detectors are the argon ionization detector, flame emission detector, cross section detector, thermal conductivity detector and the electron capture detector. Choosing the proper detector depends upon the use. Some considerations are the flame detectors destroy the sample, the thermal conductivity detector is universally sensitive, and the argon ionization detector requires argon a carrier gas. The spectral output is usually stored electronically and displayed on a monitor.

The argon ionization detector does not detect water, carbon tetrachloride, nitrogen, oxygen, carbon dioxide, carbon monoxide, ethane, or compounds containing fluorine. The flame ionization detector does not respond to water, nitrogen, oxygen, carbon dioxide, carbon monoxide, helium, or argon. If a specimen contains water, a flame ionization detector should be used. The electron capture detector cannot detect simple hydrocarbon but does detect compound containing halides, nitrogen, or phosphorus.

2.8.2 Retention Time

The amount of the time that a compound is retained in the GC column is known as the retention time. The retention time from the sample injection should be measured until the measured compound elutes from the column. The retention time can aid in differentiating between some compounds. However, identical retention times for two samples only indicate a possibility that the samples are the same substances. Potentially thousand of chemicals may have the same retention time, peak shape, and detector respond. The qualitative of their times and mass spectral with those of the library. **[10].**

2.8.3 Quality Assurance/Quality control Procedures

Before analyze a sample, technician should tune and calibrate the instrument. Tuning can be accomplishing using specific concentration of the Decafluorotriphenylphosphine and p-Bromofluorobenzene. Technician can process a spiked sample (containing a known concentration, of a substance) to check calibrating and tuning. If the GC instrument does not detect or show a greater or lesser concentration that the know concentration, Technician have to recalibrate the instrument. The blank sample (containing no detectable compound) also can be used to test the GC instrument `s data reporting accuracy.

If the device indicates the present of a substance in the blank sample, the device may contain residue from prior analysis. If this occurs, it must return and calibrate the instrument. The GC signals are converted on line to their corresponding concentration values. The recording and process data is available for process control.

2.8.4 Analysis of Output

Less than ideal spectral peaks may indicate less than idea analytical procedures or equipment. It can readily observe whether the output exhibits unsatisfactory results. Ideally, the spectral peaks should be symmetrical, narrow, separate (not overlapping), and made with smooth lines. GC evidence may be suspect if the peaks are board, overlapping, or unevenly formed. If a poorly shaped peak contains a steep front and a long, drawn-out tail, this may indicate traces of water in the specimen.

Technician should inject the specimen into the septum rapidly and smoothly to attain good separation of the components in a specimen. If the injection of the specimen too slowly, the peak may be broad or overlap. A twin peak may result from the hesitating during the injection. A smoothly performed injection, without abrupt changes, should result in a smoothly formed peak. A twin peak may also indicate that we injected two specimens consecutively.

2.8.5 Limitations

The size of a spectral peak is proportional to the amount of the substance that reaches the detector in the GC instrument. No detector responds equally to different compounds. Result using one detector will probably differ from results obtained using another detector. Therefore, comparing analytical results to tabulated experimental data using a different detector does not provide a reliable identification of the specimen.

A response factor must be calculated for each substance with a particular detector. A response factor is obtained experimentally by analyzing a known quantity

The bexperimental conditions (temperature, pressure, carrier gas flow rate) must be identical to those used to analyze the specimen. The response factor equal the area of the spectral peak divide by the weight or volume of the substance injected. If technician applies the proper techniques, of running a standard sample before and after the specimen, determining a response factor is not necessary.

2.8.6 Worm Septum

An injection port septum should last between 100 and 200 injections. Higher injection port temperature shortens the septum's lifespan. A leaking septum adversely affects the GC instrument's sensitively.

If a portion of the specimen leak back out of the septum, the amount of the specimen is not recorded. This event makes any eventual quantitative result erroneous. If air should leak into the injection port through a worm septum, the oxygen and water contained in air may skew the results. Any oxygen may react with specimen compounds. If this happens, the GC instrument will provide results indicating the presence vial. Any water in the column adversely affects the GC instrument's ability to separate components.

2.8.7 Injection Port Temperature

The temperature of the GC injection port must be high enough to vaporize a liquid specimen instantaneously. If the temperature is too low, separation is poor and broad spectral peaks should result or no peak develops at all. If the injection temperature

is too high, the specimen may decompose or change its structure. If this occurs, the GC result will indicate the presence of compounds that were not in the original specimen.

2.8.8 Residual Impurities

Ideally, all components of a specimen elute completely from the GC column. If any substance remains inside the column, the substance may elute during subsequent analyses with other specimens. This may result in an unexpected peak in the output. The peak produced should be broad.

2.8.9 Carrier Gas

If the GC instrument uses hydrogen for the carrier gas, we must consider whether the hydrogen may react with any of the compounds present in the specimen. If the hydrogen does not react, a broad peak will result. When using a thermal conductivity detector, care should be taken as a false peak may occur if the carrier gas's thermal conductivity is in the range of the thermal conductivity of any compound in the specimen. An unstable carrier gas flow rate may produce a drifting baseline and false broad peak. A carrier gas should be pure. Regular changing of the gas filter prevent significant impurities.

2.8.10 Crucial Factor

GC analysis is highly if the instrument is properly maintained, if follows proper procedures, and the interpretation of the results is competent. While some factor rarely affect GC analysis, some factors are absolutely essential for the use of reliable GC

evidence. In all case a content of all collected specimen. This standard sample must be processed before and after the collected specimen under identical conditions. Any output from the collected specimen that does not match the standard sample is inconclusive. If tabulated data exists for the relevant conditions, the specimen data must match the reference data.

If advance notice of GC testing is available, an adverse party should observe the procedure. If a retained consultant or the knowledgeable attorney technician use of the GC instrument. Important information can be recorded. Technician preparation of the specimen and subsequent injection can be observed for errors or malfunctioning equipment. The observer should record the instrument's make, model, serial number, injection temperature, column temperature, carrier gas flow rates pressure, identify the type of detector used, and observe any manipulation of the data by use of time elution. Any discrepancy in the time will produce an erroneous retention time. If the procedure cannot be observed, the adverse party should seek all pertinent information (experimental conditions, measurements, instrument identification) and hard copy output.

2.9 Mass Spectrometry (MS)

MS analysis commonly used in argon investigations, engine exhaust analysis, petroleum product analysis, and blood monitoring in surgery. MS identifies substance by electrically charging the specimen molecules, accelerating them through a magnetic field, breaking the molecules into charged fragments and detecting the different changes. A spectral plot displays a mass of each fragment. Technician can use a compound's mass spectrum for qualitative identification. We uses these fragment masses as puzzle pieces to pieces together the mass of the original molecules, the "parent mass." hey have proven sensitive in both the total ion scan as well as in the selective ion mode (SIM) **[18].**

The parent mass is analogous to the picture on top of a puzzle box, a guide to the end result obtained by putting together the fragment masses, or puzzle pieces. From he molecular mass and the mass of the fragment, reference data is compared to determine the identity of the specimen. Each substance's mass spectrum is unique. Providing that the interpretation of the output correctly determines the parent mass, MS identification is conclusive. Meanwhile, mass spectrometry detectors are far more accurate than an FID for component identification. The percent composition of an oil are obtained from the mass spectrum detector.

2.9.1 Description of Mass Spectrum Process

There are many types of MS exist, each one using different apparatus and process for producing mass spectra. Such as instrument contains a sample inlet, an ionization source, a molecule accelerator, and a detector.

MS analysis required a pure gaseous sample. The sample inlet is maintain at an high temperature, up to 400 degree C (752 degree F)**,** to ensure that the sample stays a gas [**3**]. Next the specimen enters the ionization chamber. A beam of electrons is accelerated with a high voltage. The specimen molecules are shattered into well-defined fragments upon collision with the high voltage electrons. Each fragment is charged and travels to the accelerator as an individual particle. In the acceleration chamber the charged particle's velocity increase due to the influence of an accelerating voltage. For one value of voltage only one mass accelerates sufficiently to reach the detector. The accelerating varies to cover a range of masses to that all fragment reach the detector.

The charged particles travel in a curved path towards the detector. When as individual charged particle collides with the detector surface, several electrons (also

charged particles) emit from the detector surface. Next, these electrons accelerate towards a second surface, generating more electrons, which bombard another surface. Each electron carries a charge. Eventually, multiple collisions with multiple surfaces generate thousand of electrons that emit from the last surface. The result is amplification or the original charge through a cascade of electrons arriving at the collector. At this point the instrument measures the charge and record the fragment mass as the mass is proportional to the detected charge.

The MS instrument produces the output by drawing a array of peaks on a chart, the “mass spectrum “. Each peak represents a value for a fragment mass. A peak’s height increase with the number of fragments detected with one particular mass. As in the case of the detectors, a peak may differ in height with the sensitivity of the detector used.

2.9.2 Analysis of Output

Each substance has a characteristic mass spectrum under particular controlled conditions. Technician can identify a specimen by comparing the specimen’s mass spectrum with known compounds. Quantitative analysis is possible by measuring the relative intensities of the mass spectra.

Usually a mass spectrum will display a peak for the unfrequented molecule of the specimen. This is commonly the greatest mass detected, called the “ parent mass “. Like the picture on the puzzle box, the parent mass is used to fit the pieces together from the other peaks in the mass spectrum. The parent mass of the molecule while the other peaks indicates the molecule’s structure.

Determining the parent peak and consequently the molecular mass of the specimen is the most difficult part of MS analysis. Identifying the parent mass is outside the scope of this article. Assuming that we can correctly determine the molecular mass, technician make an educated guess of the specimen's identify and compares the mass spectrum to reference for conformation. The mass spectral for larger molecules containing carbon are complicated and require tedious calculations that are subject to error. Computers are commonly used for spectral analysis.

2.10 Limitations

2.10.1 Resolution

The "Resolution" is a value that represent the instruments a ability to distinguish two particles of different masses. The greater the MS instrument's resolution, the greater its usefulness for analysis. An MS instrument provides more accurate results for larger molecules when the instrument has a high resolution. A high resolution MS instrument is advisable for analyze body fluids because they have high molecular masses. A low resolution MS instrument may not sufficiently characterize a large mass. If the interior pressure in an MS instrument is too high, erroneous results may occur. As the specimen molecule braes up, the fragment accelerates. If a fragment collides with another fragment, then these two fragments may combine to make a new particle. In this event, the detector will register the mass of this new particle on the mass spectrum. The reference spectra for comparison are produced under low-pressure conditions, which minimize collisions between fragments. Technician would find a spectral peak where one is not expected. In the puzzle analogy, this similar to finding pieces from a different puzzle in your box and trying to make these extraneous pieces fit. As this impossible, any MS analysis under high-pressure conditions would depend greatly on guesswork **[3]**.

2.10.2 Parent Mass

Finding the correct parent peak in the mass spectra may be difficult. Finding the peak helps to determine the parent mass, which should lead to determining the specimen's molecular mass. For high molecular mass compounds, like drugs and body fluids, a parent peak is often not observed. This makes qualitative identification difficult. A special type of MS, chemical ionization MS, reduce the likelihood of missing the parent mass **[3]**.

2.10.3 High Speed Scanning

High-speed scanning MS instruments are able to rapidly analyze specimens. However, the increased speed is a tradeoff for decreased resolution. Quantitative measurements are unreliable with high speed scanning.

2.10.4 Technician's Skills

As in the puzzle analogy, knowing the shape of a piece of the molecule helps to join the pieces together. To determine the specimen's molecular structure before fragmentation, the technician need to employ skill and art to determine the molecular structure from mass spectra patterns. Computers and Database can assist, but human expect is necessary to distinguish between likely and unlikely answers. Alone, a computer cannot determine molecular structures as well as a competent human. This causes the weight of MS evidence to depend greatly on the technician's qualifications and proficiently with MS spectrum analysis.

2.10.5 Crucial Factors

Technician must process a standard sample containing a verified composition identical to the presumed contents of the collected specimen. This standard sample must be processed under identical conditions, both before and after processing the collected specimen. Any identification based on output from the collected specimen that does not match the standard sample is inconclusive.

MS is highly sensitive, care should be taken that not even the slightest trace of a previous sample remain within the MS instrument. Technician should run the "background spectrum" an analysis without a specimen, before analyzing the specimen in question. This practice is the only way that an independent analysis can definitely interpret MS output.

If tabulated reference data exist for the relevant conditions, the specimen data must match the reference data. Despite the use of sophisticated instruments, computer, and proficient personnel, they're some double in conclusion based on interpretation of mass spectra. If advance notice of MS testing is available, an adverse party should observe the procedure. If a retained consultant or the knowledgeable attorney observes the technician's use of the MS instrument, important information can be recorded. The observer should record the instrument's use make, model, serial number, resolution, pressure, and identify the type of detector use.

2.11 GC/MS Combination

The GC instrument is effective in separating compounds into their various components. GC instrument cannot be used for reliable identification of the specific substance. The MS instrument provides specific result but produces uncertain qualitative results. The technician has access to both the retention times and mass spectral data.

GC/MS analysis, where the effluent to the GC instrument is the fed to the MS instrument, is in wide use for confirmation testing of substance **[3]**.

2.12 Kovat Index (KI)

Kovet index is used to calculate or to determined the number of chemical components exist in the sample of Gaharu essential oil. To determine the unknown substances, the value of kovet index (KI) will be compared with journal. The kovet index (KI) value from journal with a range of +/- 5 will be acceptable **[19].**

$$KI = \frac{100\,[\,\mathrm{Log}\,(Tx - Tm) - \mathrm{Log}\,(Tn - Tm)}{[\,\mathrm{Log}\,(Tn + 1 - Tm) - \mathrm{Log}\,(Tn - Tm)} + 100\,(N)$$

Where,

Tm = Mobile Phase retention time
Tx = Sample compounded retention time
Tn = Standard hydrocarbon containing carbon retention time
Tn+1 = The next standard hydrocarbon containing carbon retention time
N = Lowest carbon value

CHAPTER 3

METHODOLOGY

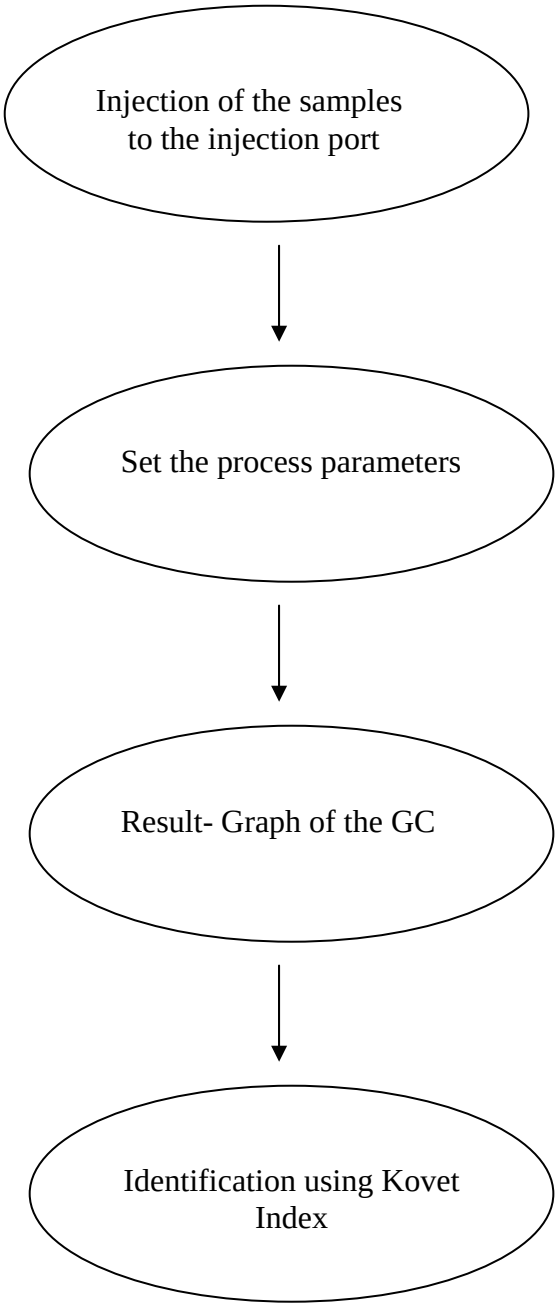
3.0 Main Process

In this research, there are two main process to measure the project were success. The process were **(1)** Separation of the substances based on the molecular weight (MW) using GC-GCMS, and **(2)** Identification of the substances detected from the separation process using the Willey Library software and compared the Kovet Index (KI) based on the retention time from process of separation with the Kovet Index of the same substances from the Journal.

3.1 Separation Process by GC-GCMS

All the 7 samples from different grade of Gaharu essential oil were analyzed and identified using the GC-GCMS.

3.1.1 Separation by Gas Chromatography (GC)



3.1.2 Separation by Gas Chromatography /Mass Spectrometry (GC/MS)

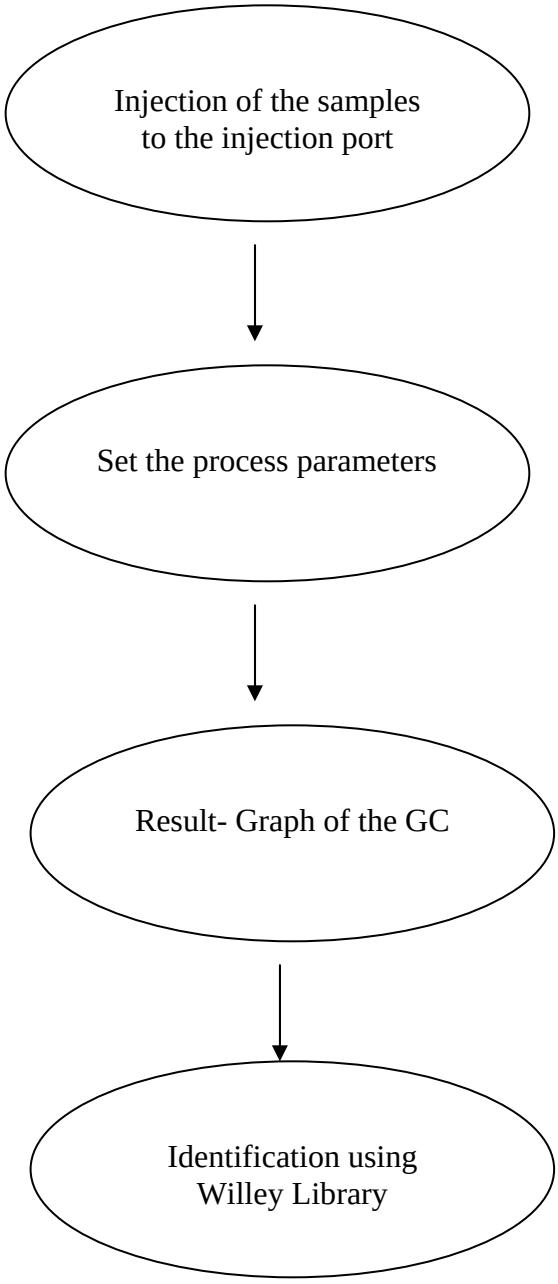


Table 3.0 :- Information of Gas Chromatography (GC)

Model	GC QP2010
Column	CBP5 (30 m x 0.25mm; 0.25 um film thickness)
Programmed	Initially at 75 C for 10 minutes and 3 C/min to 230 C for 1 min
Detector	FID

Table 3.1 :- Information of Gas Chromatography and Mass Spectrometry (GCMS)

Model	Hewlett Packard GC/MSD 5890 series II
Column	DB-1 (30 m x 0.25mm; 0.25 um film thickness)
Programmed	Programmed from 60 C for 10 minutes then rising at 3 C/min to 230 C for 1 min
Detector	FID

3.2 Identification Process

3.2.1 Willey Library

The components that can be detected from the Mass Spectrometry (MS) will be compared with the Willey Library software. The comparison between the software and the substances detected is make based on the Molecular Weight. Only the substances that have an equality of more that 50% were acceptable.

3.2.2 Kovet Index (KI)

The retention time from the GC result will be calculated to get the Kovet Index for all components that can be detected. This calculation is based on the standard Hidrocarbon (HC) that have been run using the same column for the all samples.

$$KI = \frac{100 [\text{Log} (Tx - Tm) - \text{Log} (Tn - Tm)}{[\text{Log} (Tn + 1 - Tm) - \text{Log} (Tn - Tm)} + 100 (N)$$

Where,

Tm = Mobile Phase retention time

Tx = Sample compounded retention time

Tn = Standard hydrocarbon containing carbon retention time

Tn+1 = The next standard hydrocarbon containing carbon retention time

N = Lowest carbon value

CHAPTER 4

RESULT AND DISCUSSION

4.0 Results

From the analysis, the best quality of Gaharu essential oil based on the major components (Agarospirol, Jinkoheremol and Khusenol) and the number of components exist in all samples of Gaharu essential oil.

Table 4.0: Result of analysis for sample G1

Item	Components	RT (Retention Time)	Kovet Index KI
1	2-Butanone-3-phenyl	23.642	1227
2	α –Guaiene	34.319	1450
3	β- agarofuran	35.423	1475
4	α - bulnesene	36.793	1506
5	epoxybulnese	39.688	1578
6	Guaiol	40.611	1600
7	4-hydroxy-3,5-dimethylbenzaldehyde	41.069	1612
8	1,5-epoxy -nor-ketoguaiene	41.224	1617
9	Agarospirol	41.837	1633
10	4-(4-hydroxy-3-methyphenyl-	41.932	1636
11	jinkoh eremol	42.1	1640

12	kusunol	42.455	1650
13	Selina-3,11-dien-9-one	43.923	1688
14	rotundone	44.69	1709
15	selina-3,11-dien-9-ol	45.152	1722
16	Selina,-4-11,dien-14-oic acid	45.334	1727
17	selina,3-11-dien-14-al	45.442	1730
18	9,11-eremophiladien-8-one	45.783	1740
19	selina,4-11-dien-al	46.594	1763
20	Guaia-1(10),11-dien-15-ol	46.887	1772
21	dihydrokaranone	47.695	1793
22	karanone	48.679	1811
23	oxo-agarospirol	48.679	1822
24	2-hydroxyguaia-1(10),11-dien-15-oic acid	52.647	1939
25	9-hydroxyselina-4,11-dien-14-oic acid	52.976	1949

In sample G1, there are three major components (Agarospirol, Jinkoheremol and Khusenol exist in this sample. The retention time for Agarospirol is 41.837 and the Kovet Index (KI) is 1633. For Jinkoheremol, the retention time is 42.10 and the Kovet Index (KI) is 1640. Finally, for Khusenol component the Kovet Index (KI) is 42.455 and the Kovet Index (KI) is 1650.

Table 4.1: Result of analysis for sample G2

Item	**Components**	**RT (Retention Time)**	**Kovet Index KI**
1	2-Butanone-3-phenyl	23.663	1227
2	α –Guaiene	34.326	1450
3	β- agarofuran	35.431	1475
4	α - bulnesene	36.938	1510
5	norketoagarofuran	38.677	1553

6	epoxybulnese	39.654	1577
7	Guaiol	40.592	1599
8	4-hydroxy-3,5-dimethylbenzaldehyde	41.006	1610
9	1,5-epoxy -nor-ketoguaiene	41.205	1616
10	Agarospirol	41.822	1633
11	4-(4-hydroxy-3-methyphenyl-	41.907	1635
12	jinkoh eremol	42.071	1640
13	kusunol	42.428	1649
14	Selina-3,11-dien-9-one	43.903	1687
15	rotundone	44.671	1708
16	Selina,-4-11,dien-14-oic acid	45.355	1728
17	selina,3-11-dien-14-al	45.451	1731
18	9,11-eremophiladien-8-one	45.814	1741
19	selina,4-11-dien-al	46.384	1757
20	Guaia-1(10),11-dien-15-ol	46.851	1769
21	dihydrokaranone	47.877	1798
22	Guaia-1(10),11-dien-15-al	48.01	1801
23	karanone	48.347	1812
24	2-hydroxyguaia-1(10),11-dien-15-oic acid	52.692	1940

In sample G2, there are three major components (Agarospirol, Jinkoheremol and Khusenol exist in this sample. The retention time for Agarospirol is 41.822 and the Kovet Index (KI) is 1633. For Jinkoheremol, the retention time is 42.071 and the Kovet Index (KI) is 1640. Finally, for Khusenol component the Kovet Index (KI) is 42.428 and the Kovet Index (KI) is 1649.

Table 4.2: Result of analysis for sample G3

Item	Components	RT (Retention Time)	Kovet Index KI
1	2-Butanone-3-phenyl	23.597	1226.5
2	α –Guaiene	34.072	1444.2
3	β- agarofuran	35.424	1475
4	4-hydroxy-3,5-dimethylbenzaldehyde	41.043	1611
5	1,5-epoxy -nor-ketoguaiene	41.209	1616
6	Agarospirol	41.83	1633
7	4-(4-hydroxy-3-methyphenyl-	41.946	1636
8	jinkoh eremol	42.086	1640
9	kusunol	42.381	1647
10	Selina-3,11-dien-9-one	43.905	1687
11	rotundone	44.612	1705
12	Selina,-3-11,dien-14-ol	46.156	1750
13	selina,4-11-dien-14-al	46.398	1757
14	Guaia-1(10),11-dien-15-ol	46.824	1769
15	dihydrokaranone	47.867	1798
16	Guaia-1(10),11-dien-15-al	48.293	1810
17	palmitic acid	51.837	1914

In sample G3, there are three major components (Agarospirol, Jinkoheremol and Khusenol exist in this sample. The retention time for Agarospirol is 41.83 and the Kovet Index (KI) is 1633. For Jinkoheremol, the retention time is 42.086 and the Kovet Index (KI) is 1640. Finally, for Khusenol component the Kovet Index (KI) is 42.381 and the Kovet Index (KI) is 1647.

Table 4.3: Result of analysis for sample SP001

Item	Components	RT (Retention Time)	Kovet Index KI
1	2-Butanone-3-phenyl	23.556	1225.6
2	α-bulnesene	36.852	1508
3	nor-ketoagarofuran	38.66	1553
4	epoxybulnesene	39.607	1576
5	4-hydroxy-3,5-dimethoxybenzaldehyde	41.101	1613
6	Agarospirol	41.775	1631
7	4(4-hydroxy-3-methoxyphenyl)-	41.963	1635
8	jinkoh-eremol	42.372	1648
9	kusunol	42.648	1655
10	selina-3,11-dien-9-one	43.857	1686
11	selina-3,11-dien-9-ol	45.252	1725
12	selina-3,11-dien-14-al	45.559	1734
13	guaia-1(10),11-dien-15-ol	46.785	1768
14	sinenofuranol	47.18	1779
15	dihydrokaranone	47.79	1796
16	2-hydroguaia-1(10),11-dien_oic acid	52.502	1933

In sample SP001, there are three major components (Agarospirol, Jinkoheremol and Khusenol) exist in this sample. The retention time for Agarospirol is 41.775 and the Kovet Index (KI) is 1631. For Jinkoheremol, the retention time is 42.372 and the Kovet Index (KI) is 1648. Finally, for Khusenol component the Kovet Index (KI) is 42.648 and the Kovet Index (KI) is 1655. From the analysis, sample SP001 is not pure Gaharu essential oil.

Table 4.4: Result of analysis for sample SP002

Item	Components	RT (Retention Time)	Kovet Index KI
1	2-Butanone-3-phenyl	23.549	1225
2	α –Guaiene	33.784	1438
3	P-methoxybenzylacetone	34.749	1460
4	β- agarofuran	35.421	1475
5	α - bulnesene	36.853	1508
6	nor-ketoagarofuran	38.642	1553
7	epoxybulnese	39.785	1580
8	Guaiol	40.508	1597
9	Agarospirol	41.763	1631
10	4-(4-hydroxy-3-methyphenyl-	41.976	1633
11	kusunol	42.656	1655
12	dehydrojinkoh-eremol	43.276	1671
13	selina-3,11-dien-9-one	43.976	1689
14	selina-3,11-dien-14-al	45.559	1734
15	9,11-eremophiladien-8-one	45.858	1742
16	selina-3,11-dien-14-ol	46.092	1749
17	guaia-1(10),11-dien-15-ol	46.731	1767
18	sinenofuranol	47.192	1779
19	dihydrokaranone	47.789	1789
20	Guaia-1(10),11-dien-15-al	48.045	1803
21	2-hydroguaia-1(10),11-dien_oic acid	52.498	1930

In sample SP002, only two major components (Agarospirol and Khusenol exist in this sample. The retention time for Agarospirol is 41.763 and the Kovet Index (KI) is

1631. Finally, for Khusenol component the Kovet Index (KI) is 42.656 and the Kovet Index (KI) is 1655.

Table 4.5: Result of analysis for sample SP003

Item	Components	RT (Retention Time)	Kovet Index KI
1	β–myrecene	8.963	971.8
2	p-methylanisol	10.985	1008.2
3	linelene	11.244	1013
4	acetophenone	12.696	1041.1
5	benzeneethanol	15.556	1083
6	benzylacetate	19.288	1150.7
7	P-methoxypheol	22.050	1195.6
8	Benzylocetone	22.786	1209.5
9	Linalyl acetate	24.210	1238.9
10	1-ethyl-2,2,6-trimethyleyclohezeane	29.270	1337
11	α –Guaiene	33.888	1440
12	P- Methoxyhenzylacetone	34.841	1461.5
13	β- agarofuran	35.579	1478.6
14	α - bulnesesene	36.872	1508
15	1,2 - Benzenedicarbexyhic acid, diethyles	38.102	1538.9
16	epoxybulnesere	39.703	1578.4
17	Guaiol	40.665	1601.2
18	1,5-epoxy -nor-ketoguaiene	41.216	1616.3
19	Agarospirol	41.810	1632.5
20	4-(4-hydroxy-3-methyphenyl-	41.932	1635.8
21	jinkoh eremol	42.232	1643.8
22	Kusonol	42.402	1648.4
23	Selina-3,11-dien-9-one	43.682	1681.8

24	Selina,-3-11,dien-14-ol	45.656	1736.4
25	9,11-eremophiladien-8-one	45.772	1739.7
26	Selina,3,11-dien-14-01	46.189	1751.4
27	Guaia-1(10),11-dien-15-01	46.830	1769.3
28	Sinenofuranol	47.052	1775.5
29	dihydrokaranone	47.807	1796
30	Guaia-1(10),11-dien-15-al	47.990	1801
31	karanone	48.338	1812
32	guaia -1(10),11-dien-15-oic acid	48.432	1814.4
33	palmitic asid	51.887	1915
34	2-hydroxyguaia - (10),11-dien-15 oic acid	52.500	1934

In sample SP003, there are three major components (Agarospirol, Jinkoheremol and Khusenol) exist in this sample. The retention time for Agarospirol is 41.810 and the Kovet Index (KI) is 1633. For Jinkoheremol, the retention time is 42.232 and the Kovet Index (KI) is 1644. Finally, for Khusenol component the Kovet Index (KI) is 42.402 and the Kovet Index (KI) is 1650. From the analysis, sample SP003 is not pure Gaharu essential oil.

Table 4.6: Result of analysis for sample SP004

Item	Components	RT (Retention Time)	Kovet Index KI
1	2-Butanone,3-phenyl	23.715	1225
2	α-bulnesene	36.809	1507
3	nor-ketoagarofuran	38.69	1554
4	epoxybulnesene	39.678	1578
5	4-hydroxy-3,5-dimethoxybenzylaldehyde	41.112	1614
6	1,5-epoxy-nor-ketoguaiene	41.293	1616

7	Agarospirol	41.736	1628
8	4-(4-hydroxy-3-methoxyphenyl	42.045	1639
9	jinkoh eremol	42.215	1643
10	kusunol	42.379	1648
11	dehydrojinkoh eremol	43.527	1678
12	selina-3,11-dien-9-one	43.958	1689
13	selina-3,11-dien-9-ol	45.121	1721
14	selina-3,11-dien-14-al	45.622	1735
15	9,11-eremophiladien-8-one	45.786	1740
16	guaia-1(10),11-dien-9-one	46.244	1753
17	selina-4,11-dien-14-al	46.514	1760
18	guaia-1(10),11-dien-15-ol	46.89	1771
19	dihydrokaranone	47.761	1795
20	guaia-1(10),11-dien-15-al	48.079	1804
21	karanone	48.559	1818

In sample SP004, there are three major components (Agarospirol, Jinkoheremol and Khusenol) exist in this sample. The retention time for Agarospirol is 41.736 and the Kovet Index (KI) is 1628. For Jinkoheremol, the retention time is 42.215 and the Kovet Index (KI) is 1643. Finally, for Khusenol component the Kovet Index (KI) is 42.379 and the Kovet Index (KI) is 1648.

4.1 Discussion on Finding

4.1.1 Sample G1

From the analysis on sample G1, three (3) major components that contributed to the special aroma to Gaharu essential oil (Agarospirol, Jinkoheremol and Khusenol) can be detected in this sample. From the analysis, the percentage area of Agarospirol is 10.1444% and the percentage of concentration is 10.2345%. The second major components, Jinkoheremol also can be detected in this sample. The percentage area of Jinkoheremol is 2.614% and the percentage concentration is 2.6375%. The percentage area of khusenol in this sample is 3.9533% and the percentage concentration is 3.9885%. Beside that, the number of components that can be detected in this sample is about 25 components.

4.1.2 Sample G2

From the analysis on sample G2, three major components also can be detected in this sample of Gaharu essential oil. From the analysis, the percentage area of Agarospirol is 5.1072% and the percentage concentration is 5.0444%. The second important components, Jinkoheremol also exist in this sample. The percentage area of Jinkoheremol in this sample is 2.1180% and the percentage concentration is 2.0918%. For the Kusenol, the percentage area of this component in G2 sample is 1.8526% and the percentage of concentration is 1.8296%. Beside that, the number of components that can be detected in this sample is only 24.

4.1.3 Sample G3

From the analysis on sample G3, three (3) major components that contributed to the special aroma to Gaharu essential oil (Agarospirol, Jinkoheremol and Khusenol) can be detected in this sample. From the analysis, the percentage area of Agarospirol is 11.1028% and the percentage of concentration is 11.0765%. The second major components, Jinkoheremol also can be detected in this sample. The percentage area of Jinkoheremol is 3.05159% and the percentage concentration is 3.0455%. The percentage area of khusenol in this sample is 4.8564% and the percentage concentration is 4.8449%. Beside that, the number of components that can be detected in this sample is only 17 components.

4.1.4 Sample SP001

From the analysis on sample SP001, three (3) major components that contributed to the special aroma to Gaharu essential oil (Agarospirol, Jinkoheremol and Khusenol) can be detected in this sample. From the analysis, the percentage area of Agarospirol is 34.275% and the percentage of concentration is 32.4581%. The second major components, Jinkoheremol also can be detected in this sample. The percentage area of Jinkoheremol is 4.1301% and the percentage concentration is 4.4564%. The percentage area of khusenol in this sample is 5.036% and the percentage concentration is 4.7689%. Beside that, the number of components that can be detected in this sample is only 16 components. From the GC/GCMS analysis, SP001 sample is not pure Gaharu essential oil. This is because, in this sample also have some components that surely is not in Gaharu essential oil such as Cyclohexane,1-methyl-4-(1-methylethyl). Beside that, strongly evident that to proof ed this sample is not pure Gaharu is because from the GC graph, the components is not dominant at the middle side. For pure Gaharu essential oil, the components must be dominant at the middle side (reffer appendix).

4.1.5 Sample SP002

From the analysis on sample SP002, only 2 (Two) major components that contributed to the special aroma to Gaharu essential oil (Agarospirol, and Khusenol) can be detected in this sample. From the analysis, the percentage area of Agarospirol is 19.6237% and the percentage of concentration is 19.6163%. The percentage area of khusenol in this sample is 3.8567% and the percentage concentration is 3.8529%. Beside that, the number of components that can be detected in this sample is only 21 components.

4.1.6 Sample SP003

From the analysis on sample SP003, three (3) major components that contributed to the special aroma to Gaharu essential oil (Agarospirol, Jinkoheremol and Khusenol) can be detected in this sample. From the analysis, the percentage area of Agarospirol is 3.8591% and the percentage of concentration is 4.3235%. The second major components, Jinkoheremol also can be detected in this sample. The percentage area of Jinkoheremol is 3.6391% and the percentage concentration is 4.0771%. The percentage area of khusenol in this sample is 7.4362% and the percentage concentration is 8.3312%. Beside that, the number of components that can be detected in this sample is only 34 components. From the GC/GCMS analysis, SP003 sample is not pure Gaharu essential oil. This is because, in this sample also have some components that surely is not in Gaharu essential oil such as linelene. Beside that, strongly evident that to proofed this sample is not pure Gaharu is because from the GC graph, the components is not dominant at the middle side. For pure Gaharu essential oil, the components must be dominant at the middle side (see appendix).

4.1.7 Sample SP004

From the analysis on sample SP004, three (3) major components that contributed to the special aroma to Gaharu essential oil (Agarospirol, Jinkoheremol and Khusenol) can be detected in this sample. From the analysis, the percentage area of Agarospirol is 15.6769% and the percentage of concentration is 15.677%. The second major components, Jinkoheremol also can be detected in this sample. The percentage area of Jinkoheremol is 4.5527% and the percentage concentration is 4.5528%. The percentage area of khusenol in this sample is 0.7664% and the percentage concentration is 0.7665%. Beside that, the number of components that can be detected in this sample is only 21 components.

CHAPTER 5

CONCLUSION

5.0 Conclusion

From the analysis that have been done on 7 samples of Gaharu essential oil (G1, G2, G3, SP001, SP002, SP003 and SP004), the best quality of Gaharu essential oil is sample G1. This is because in sample G1, all three important major components (Agarospirol, Jinkoheremol and Khusenol) exist in this sample. For sample SP002, there are only two components can be detected. Beside that, the percentage area and the percentage concentration agarospirol, jinkoh eremol and khusenol is higher that in sample G2, SP002 and SP004. Beside that, the number of components that can be detected in this sample also is more than in sample G3, SP002 and SP004. For sample SP001 and SP003, it is not a pure Gaharu essential oil based on the GC/GCMS graph and analysis than have been done. After considered the existing of major components, the percentage area and concentration of major components and the number of components can be detected in all sample, the best quality of gaharu essential oil is sample G1.

5.1 Recommendation

From the analysis, the sample of G1 is the best quality of Gaharu essential oil based on chemical consistuent. As a recommendation, in order to determined the best process of extraction that capable to produce the best quality of Gaharu essential oil, all the sample of Gaharu essential oil from different process can be compared with the chemical consistuent from the sample G1. in future, industry can used the best process of extraction to produces the best quality of Gaharu essential oil

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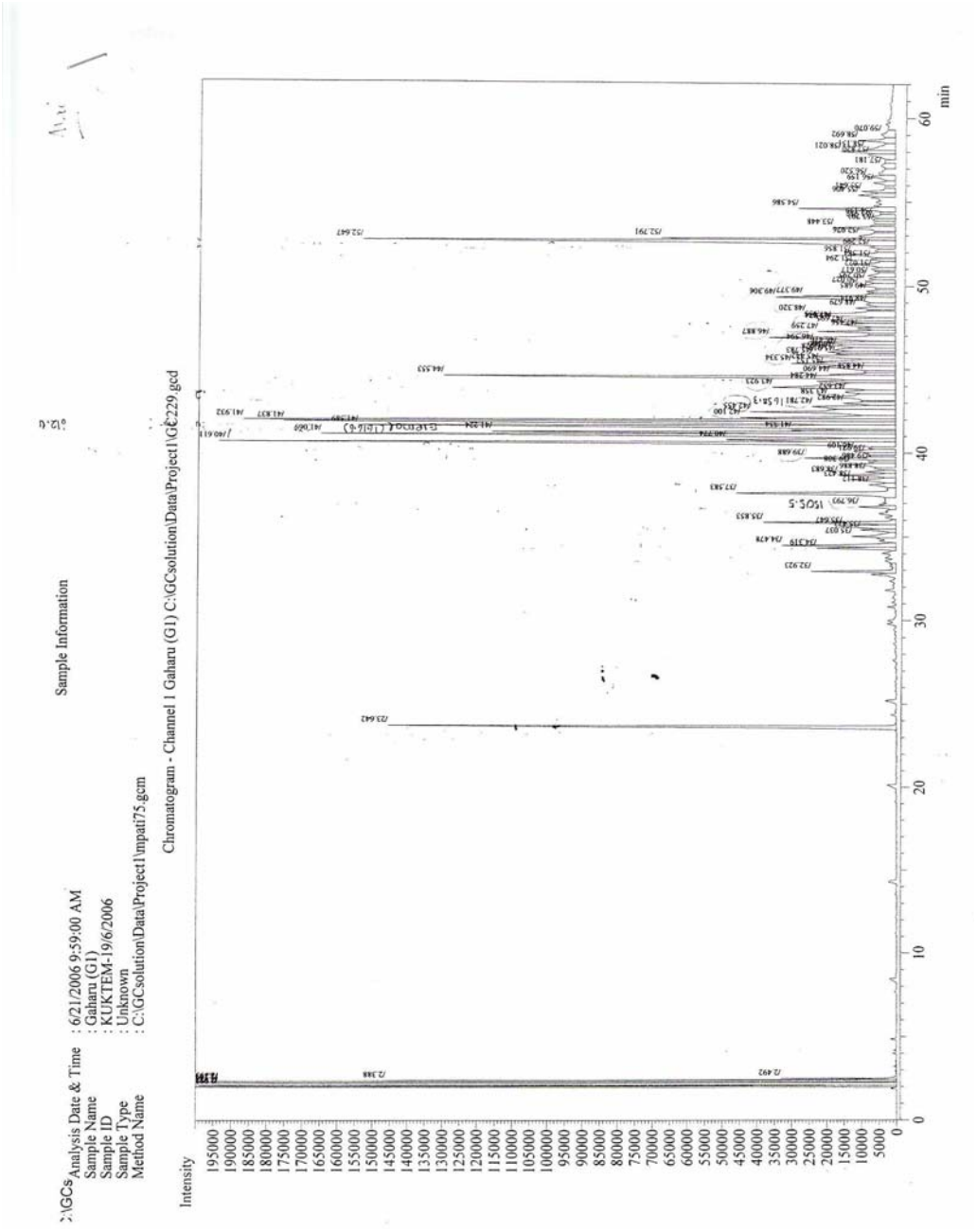
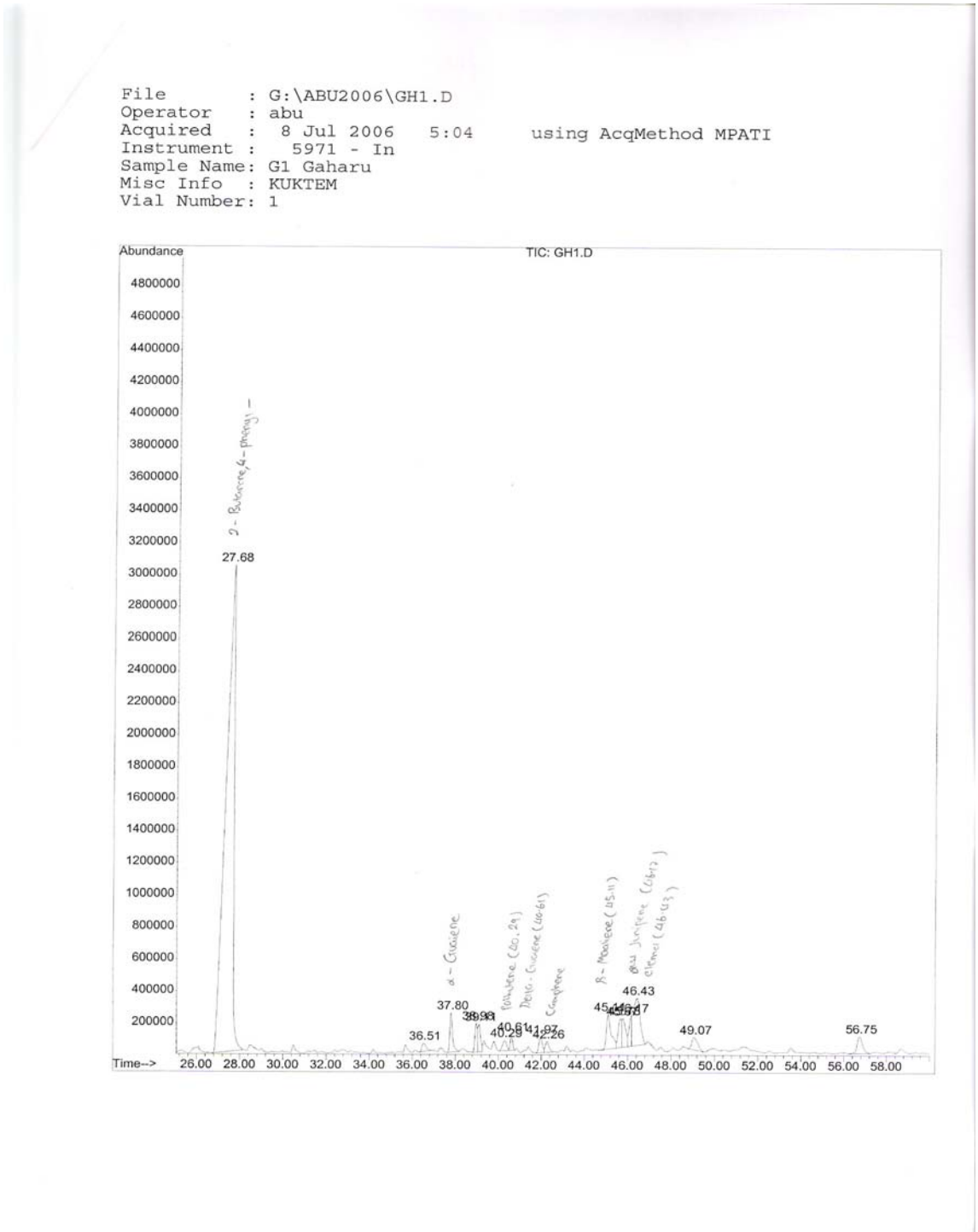
19) Masakazu Ishihara and Tomoyuki Tsuneya- Components of the Agarwood smoke on heating.

Appendix

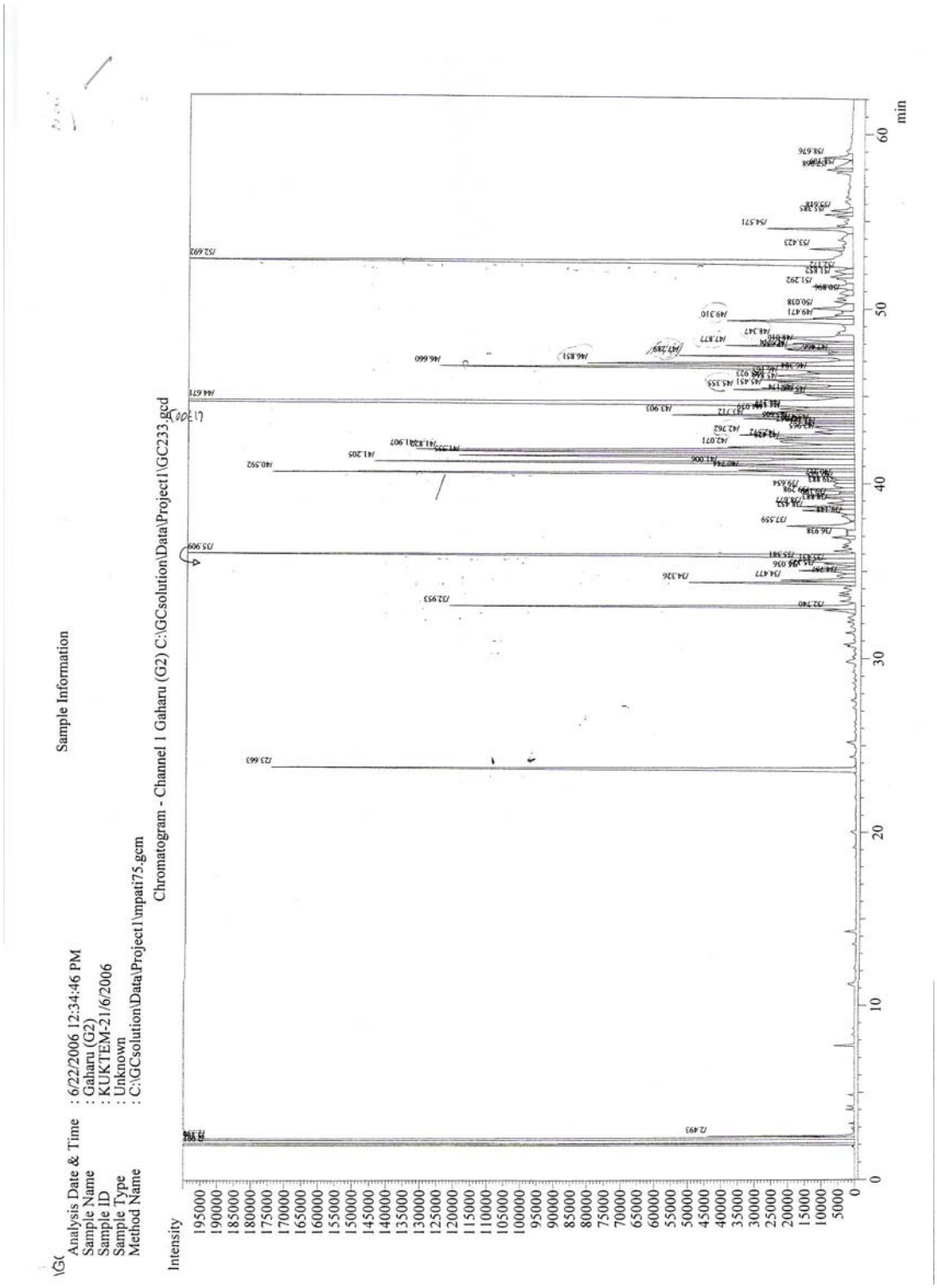
Appendix B-1 : GC Graph for sample G1



Appendix B-2 : GC/GCMS Graph for sample G1

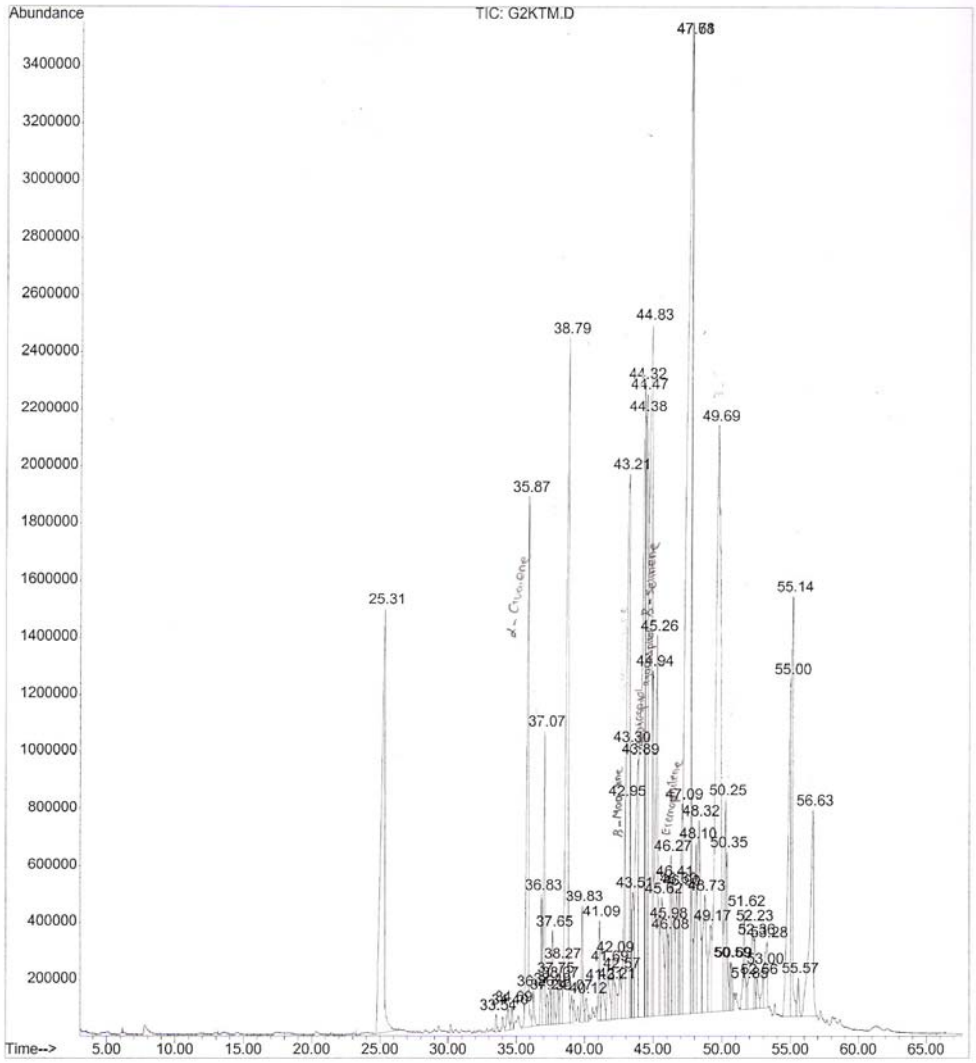


Appendix B-3 : GC Graph for sample G2



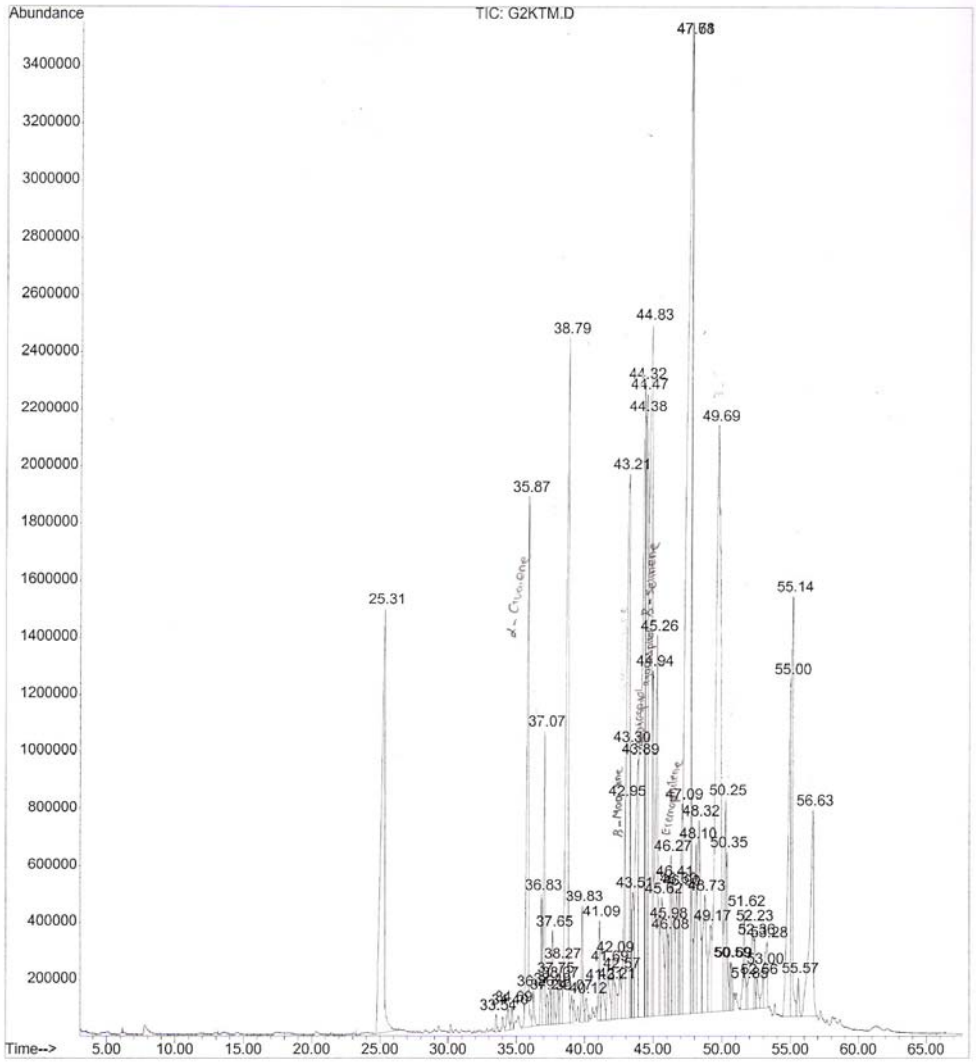
Appendix B-4 : GC/GCMS Graph for sample G2

```
File        : G:\ABU2006\G2KTM.D
Operator    : abu
Acquired    : 16 Jul 2006    8:15      using AcqMethod MPATI
Instrument :    5971 - In
Sample Name: G2
Misc Info   : Gahau G2 KUKTEM DB1
Vial Number: 1
```

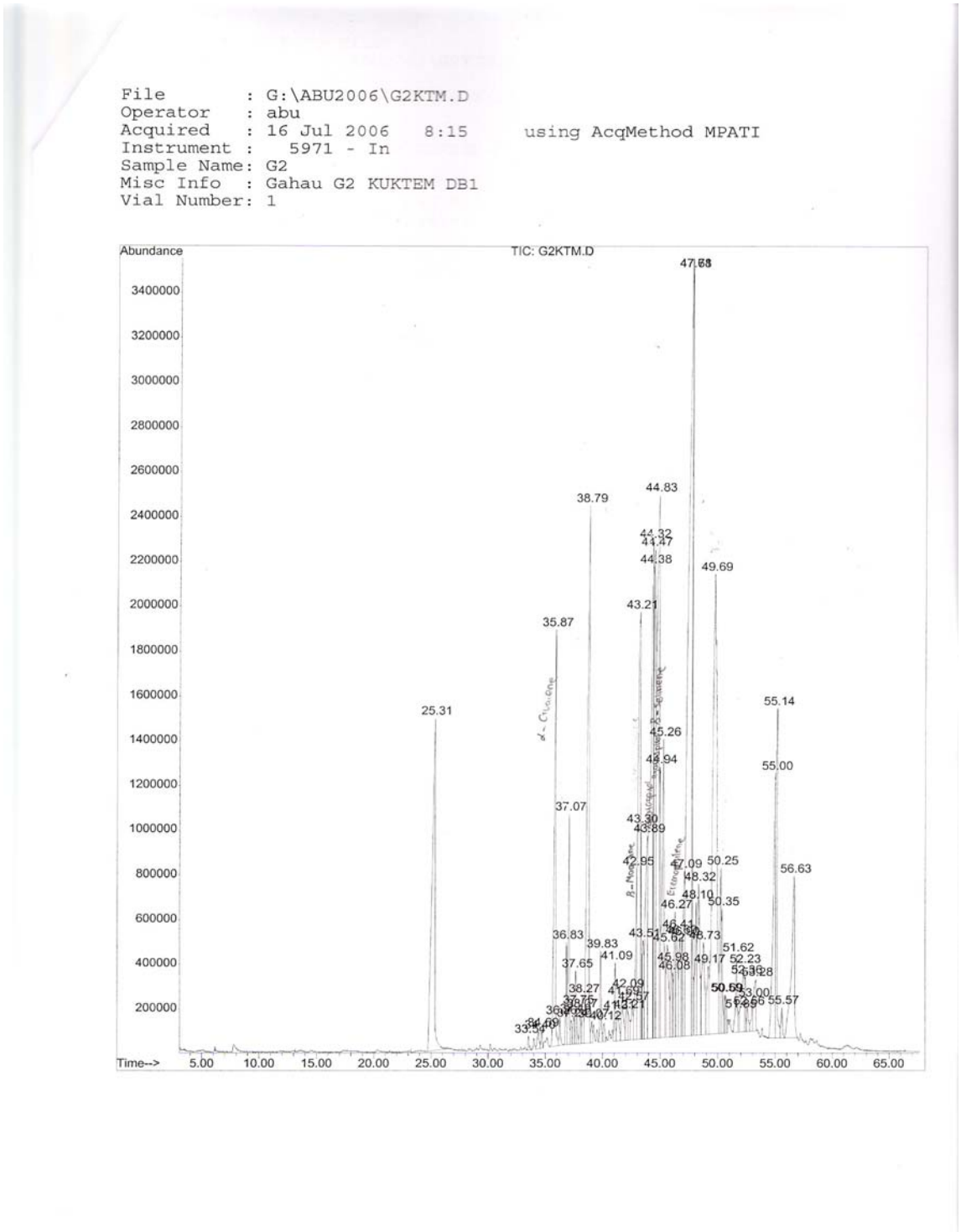


Appendix B-5 : GC Graph for sample G

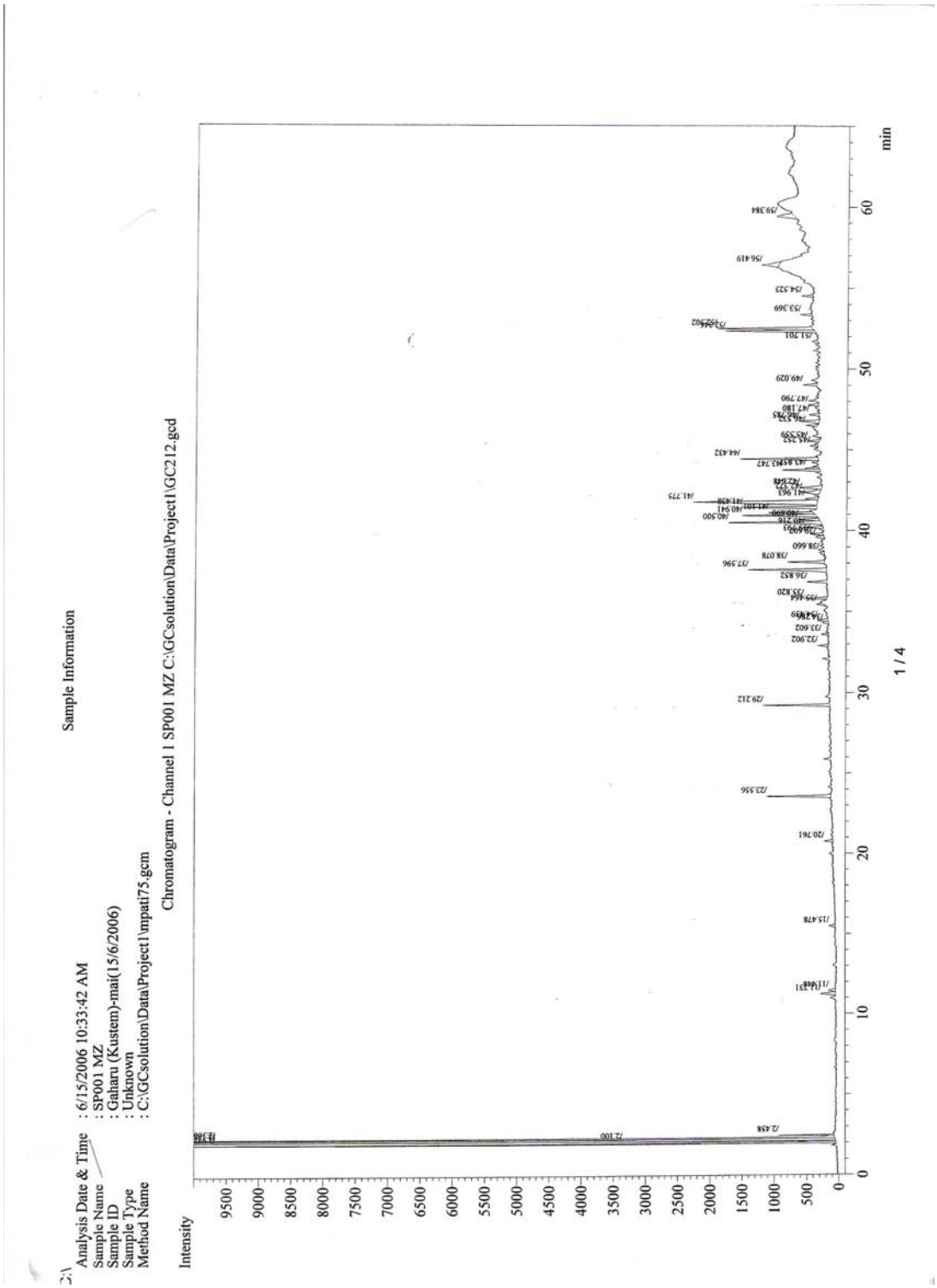
```
File        : G:\ABU2006\G2KTM.D
Operator    : abu
Acquired    : 16 Jul 2006    8:15      using AcqMethod MPATI
Instrument :    5971 - In
Sample Name: G2
Misc Info   : Gahau G2 KUKTEM DB1
Vial Number: 1
```



Appendix B-6 : GC/MS Graph for sample G3

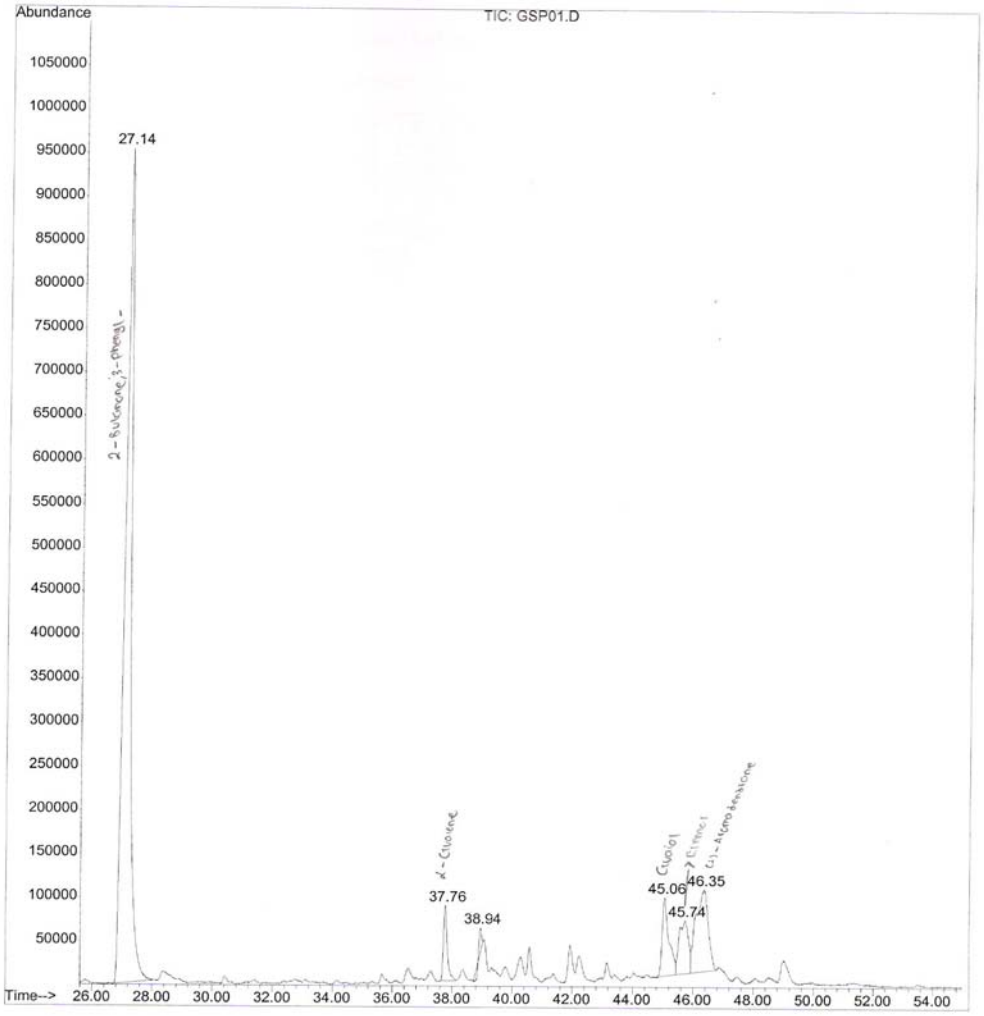


Appendix B-7 : GC Graph for sample SP001

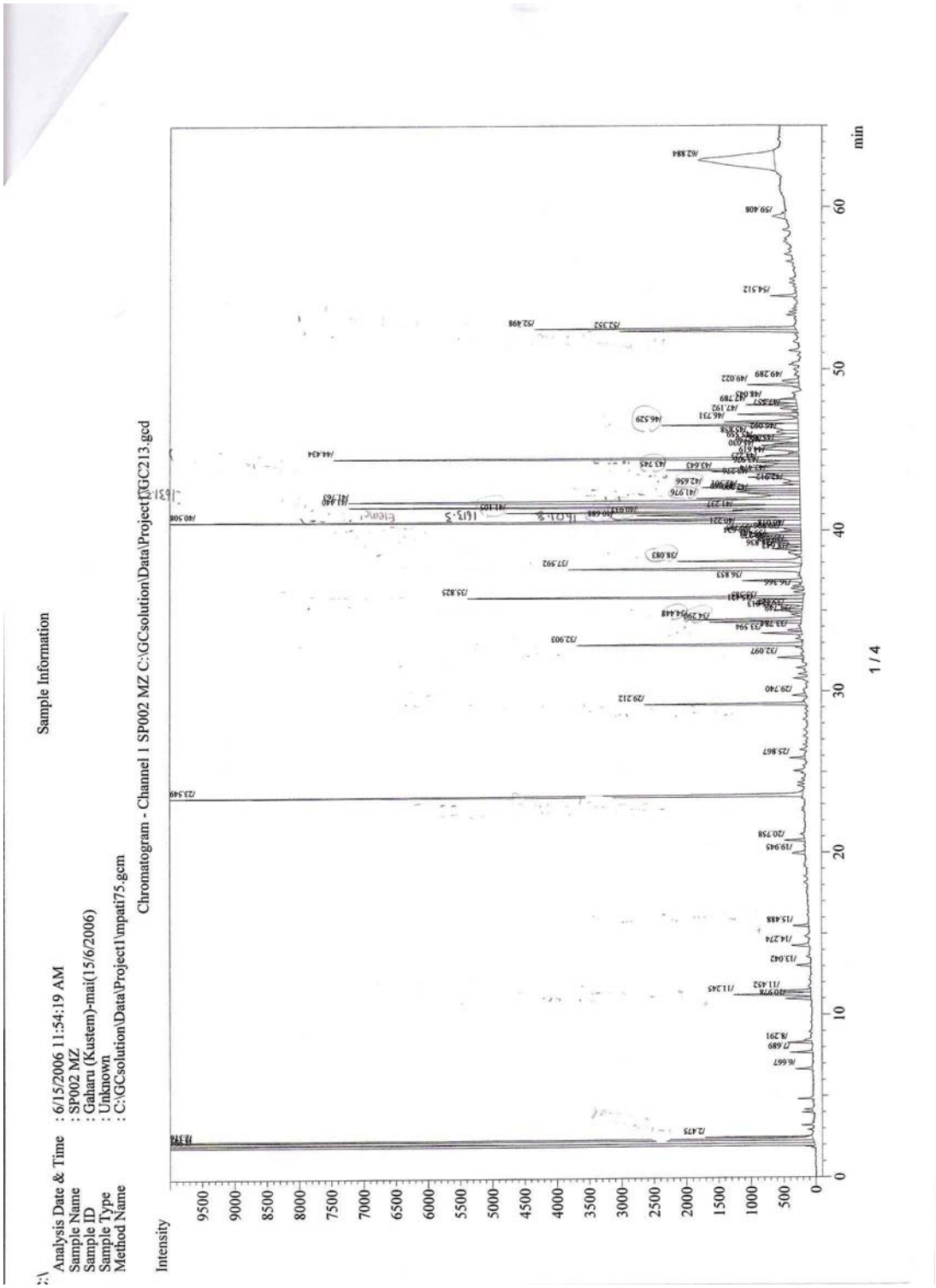


Appendix B-8 : GC/GCMS Graph for sample SP001

```
File        : G:\ABU2006\GSP01.D
Operator    : abu
Acquired    :  4 Jul 2006   8:23      using AcqMethod MPATI
Instrument  :   5971 - In
Sample Name: Gaharu sp001
Misc Info   : KUKTEM
Vial Number: 1
```

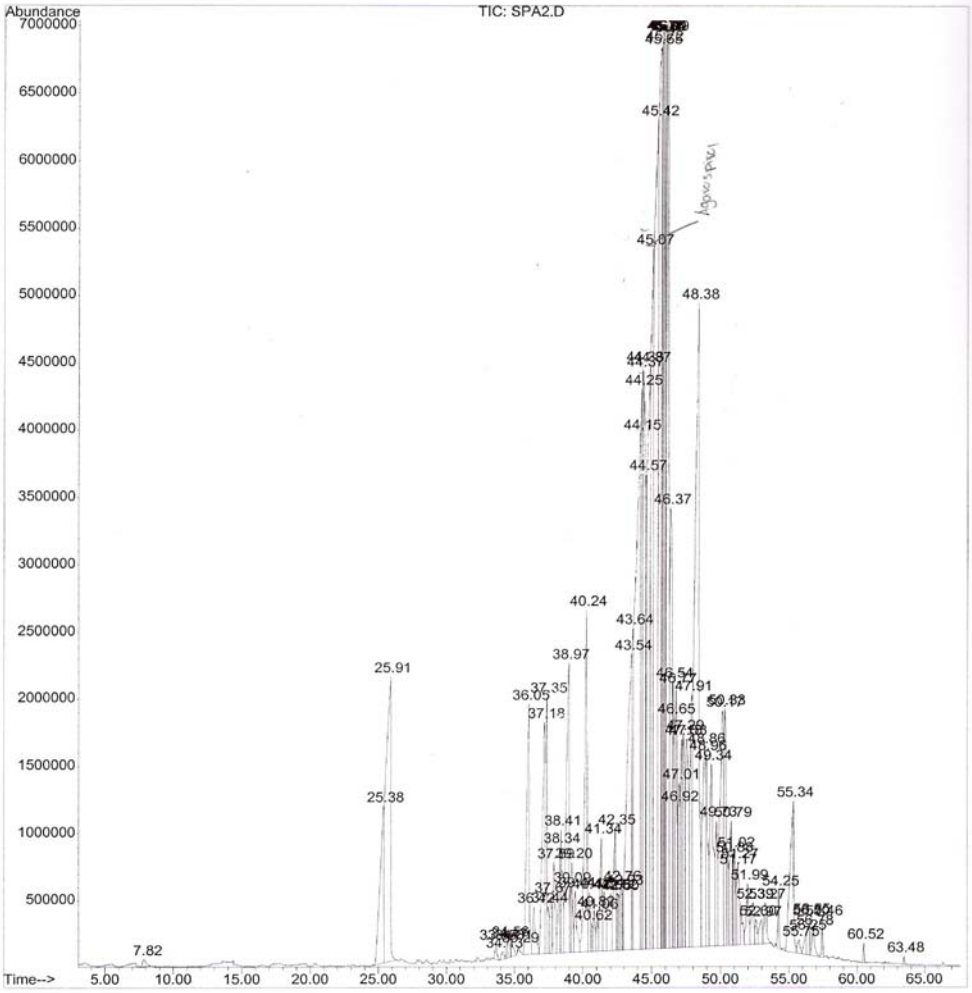


Appendix B-9 : GC Graph for sample SP002

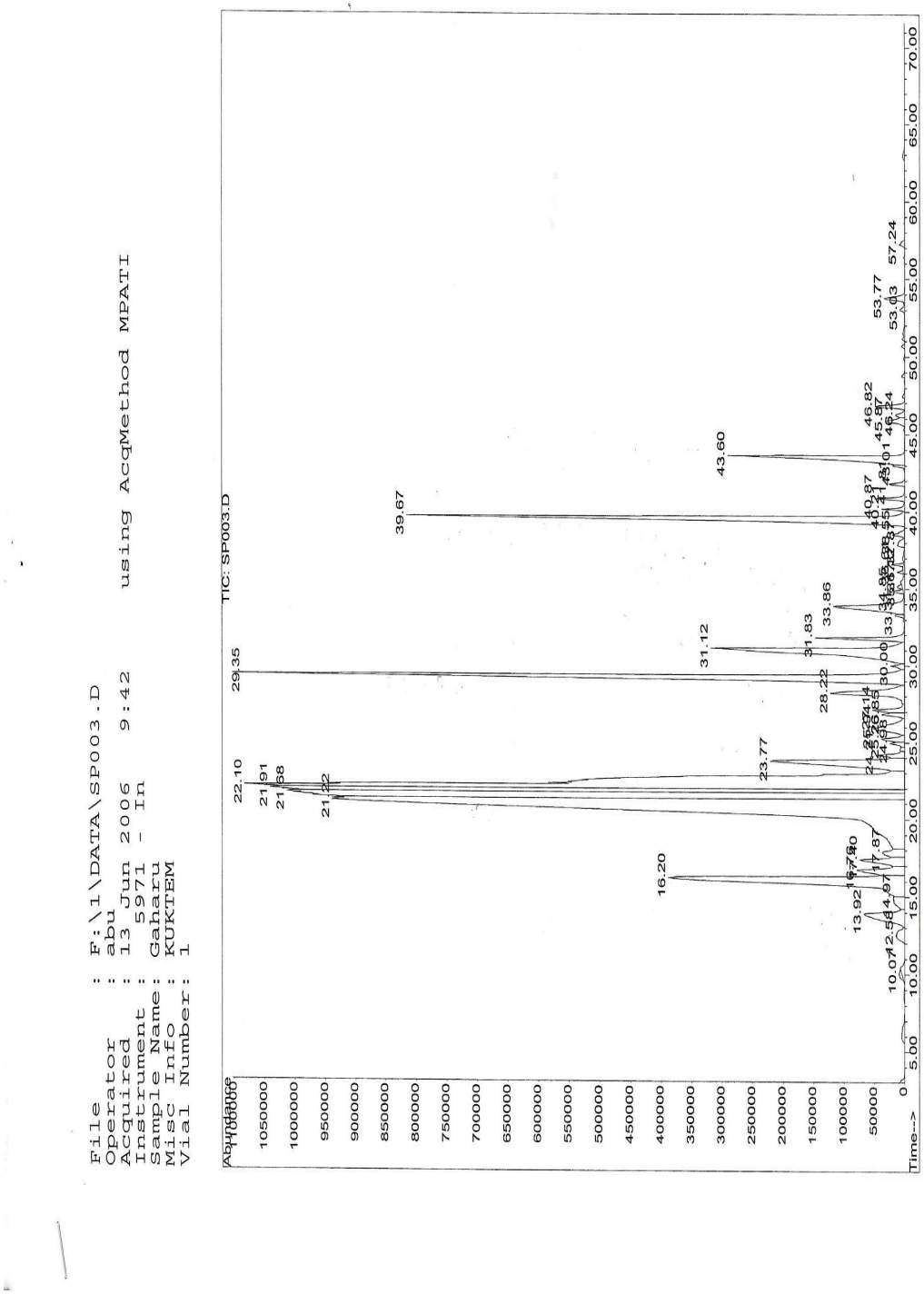


Appendix B-10 : GC/GCMS Graph for sample SP002

```
File        : G:\ABU2006\SPA2.D
Operator    : abu
Acquired    : 17 Jul 2006    7:53      using AcqMethod MPATI
Instrument  :    5971 - In
Sample Name: Gaharu SP002
Misc Info   : DB1 KUKTEM
Vial Number: 1
```

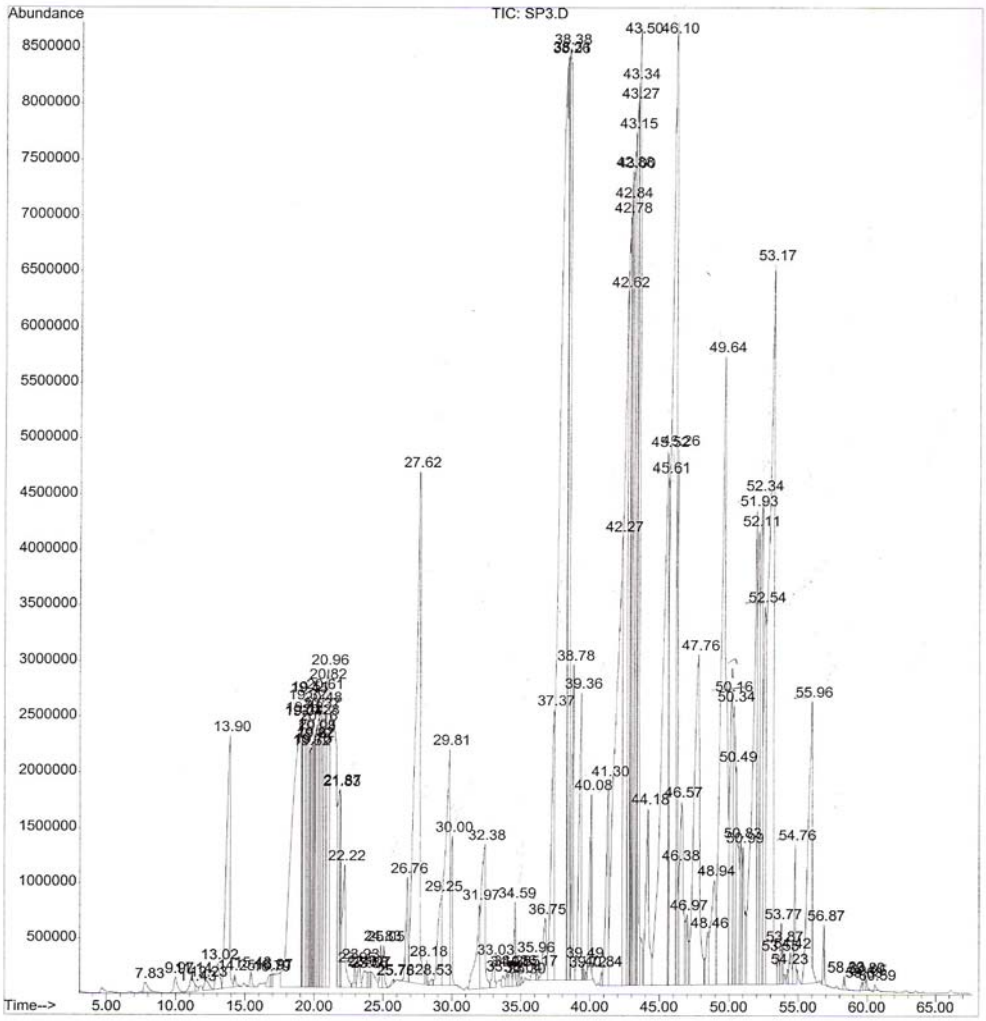


Appendix B-11 : GC Graph for sample SP003

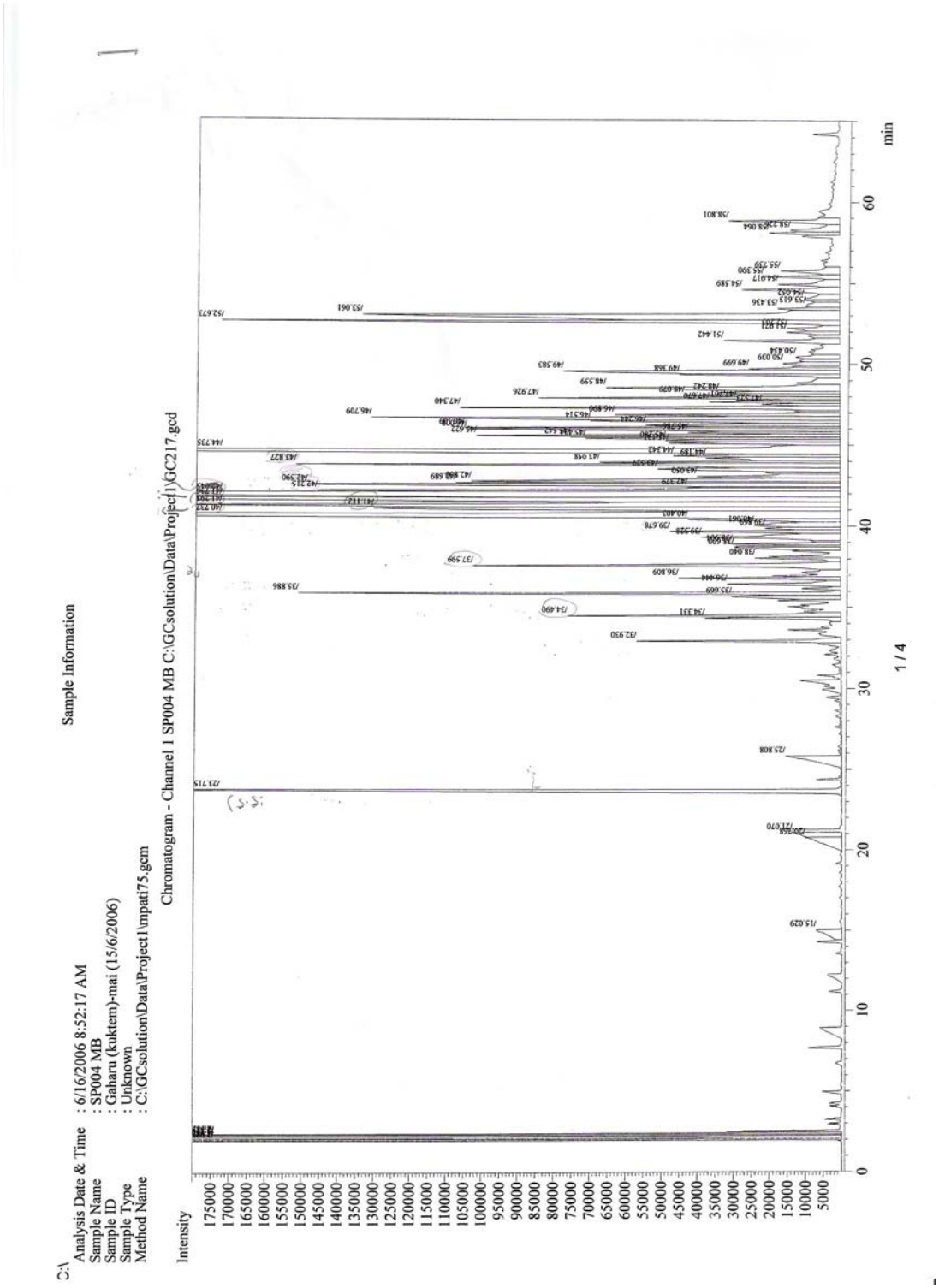


Appendix B-12 : GC/GCMS Graph for sample SP003

```
File        : G:\ABU2006\SP3.D
Operator    : abu
Acquired    : 17 Jul 2006  11:02      using AcqMethod MPATI
Instrument :   5971 - In
Sample Name: Gaharu SP003
Misc Info   : DB1 KUKTEM
Vial Number: 1
```



Appendix B-13 : GC Graph for sample SP004



Appendix B-14 : GC/GCMS Graph for sample SP004