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HIGH SENSITIVITY DIFFERENTIAL SCANNNING CALORIMETER (HSDSC) TECHNIQUE FOR ASSAYING GINGER OLEORESIN

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ABSTRACT

Thermal analysis that is DSC 'heat-flux' type was studied as additional or supportive technique to the analytical technique commonly used such as chromatographic, spectroscopic and combination of chromatographic-spectroscopic. Two medicinal plants of Zingiberaceae, that is *Zingiber officinale* Roscoe (ginger) and *Zingiber zerumbet* Smith (wild ginger) were investigated. The dried rhizomes of ginger were extracted using solvent extraction techniques to produce crude product (oleoresin) while the fresh rhizomes of wild ginger were extracted using hydrodistillation to produce the essential oil. The major compounds were separated and purified by means of several chromatographic techniques including vacuum column chromatography, gravity column chromatography and preparative thin layer chromatography. The structures were elucidated using spectroscopic methods including ^{1}H NMR, ^{13}C NMR, IR, GC and GC-MS. Two pure compounds were isolated from *Z. officinale* oleoresin and identified as 6-gingerol (brownish viscous liquid) and 6-shogaol (yellowish viscous liquid), while zerumbone (white crystals) was isolated from *Z. zerumbet* essential oil. The crude product as well as the pure compounds were analysed by using DSC technique. This method can be used to identify the major compound in crude product based on qualitative investigation of thermal behaviour (melting point, T_m and heat of fusion, ΔH_f). DSC can also estimate the purity of a substance based on the shape and temperature of the DSC melting endotherm. Sharp peak indicated pure compound while broad peak showed a mixture of various compounds.

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ABSTRAK

Analisis terma iaitu DSC jenis ‘heat-flux’ adalah dikaji sebagai teknik tambahan atau sokongan di samping teknik-teknik analisis yang selalu digunakan seperti kromatografi, spektroskopi dan gabungan kromatografi-spektroskopi. Dua spesies Zingiberaceae iaitu *Zingiber officinale* Roscoe dan *Zingiber zerumbet* Smith telah dikaji. Pengekstrakan menggunakan pelarut dijalankan ke atas rizom halia kering bagi mendapatkan ekstak mentah (oleoresin), manakala pengekstrakan rizom segar dilakukan dengan menggunakan teknik penyulingan hidro bagi menghasilkan minyak pati. Pemisahan dan penulenan sebatian kimia utama dilakukan dengan menggunakan pelbagai teknik kromatografi, antaranya kromatografi turus vakum, kromotografi turus graviti dan kromatografi lapisan nipis penyediaan. Sebatian tulen yang berjaya dipisahkan telah dikenal pasti strukturnya melalui pelbagai teknik spektroskopi iaitu RMN ^{1}H, RMN ^{13}C, IM, KG dan KG-SJ. Dua sebatian tulen utama telah berjaya dipisahkan daripada Z. *officinale* iaitu 6-gingerol (cecair pekat kuning gelap) dan 6-shogaol (cecair pekat kuning muda), manakala zerumbone (kristal putih) adalah sebatian utama di dalam minyak pati dan ekstrak mentah daripada Z. *zerumbet*. Ekstrak mentah dan sebatian-sebatian tulen tersebut dianalisa menggunakan teknik DSC. Teknik ini dapat mengenalpasti sebatian utama di dalam ekstrak mentah berdasarkan sifat-sifat termanya (takat lebur, T_m dan perubahan entalpi, ΔH_f). DSC juga mampu menganggarkan ketulenan sesuatu bahan berdasarkan keadaan bentuk dan suhu takat lebut puncak endotermik tersebut. Puncak tajam menunjukan sebatian tulen manakala puncak lebar menunjukan campuran sebatian-sebatian utama.

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NOMENCLENTURES / SYMBOLS

ΔH	changes of enthalpy
ΔH_f	heat of fusion
μW	micro Watt
^{13}C NMR	Nucleur Magnetic Resonance Carbon
^{1}H NMR	Nucleur Magnetic Resonance Proton
C	Carbon
CCl_4	carbon tetrachloride
$CDCl_3$	Deuterium chloroform
$CHCl_3$	Chloroform
d	Double
dd	double of doublet
DSC	Differential Scanning Calorimetry
DTA	Differential Thermal Analysis
Et_2O	diethyl ether
EtOAc	ethyl acetate
FID	Flame Ionisation Detector
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectroscopy
H	Hydrogen
HPLC	High Performance Liquid Chromatography
Hz	Hertz
i.d	internal diameter
IR	Infrared
KBr	Potassium bromide
m	Multiplet
mg	Milligram
MHz	Megahertz
mJ	MilliJoule
mL	Millilitre
MS	Mass Spectroscopy

NaCl	Sodium chloride
OH	Hydroxide
PE	petroleum ether
R	gas constant
R_f	retardation time
RI	Refractive Index
s	Singlet
t	Triplet
T	absolute temperature
T_0	melting point of a theoretical sample with zero impurity
T_e	end temperature / peak end
T_i	initial temperature / peak start
TLC	Thin Layer Chromatography
T_m	melting temperature of sample
TMS	Tetramethylsilane, $(CH_3)_4$ Si
T_p	peak maximum temperature
UV	Ultra Violet
x	mole fraction

LIST OF APPENDICES

GLOSSARY

Analyte	The species in the sample about which analytical information is sought
Anti-emetic	A substance to prevent vomiting
Anti-rheumatic	A substance used to alleviate rheumatism (pain, swelling and deformating of joints)
Authentic sample	Isolated sample obtained from previous research which had already determined as pure
Calibration	The process of ensuring that the signal measured by an instrument is correct
Chromatogram	A plot of analyte signal as a function of elution time or elution volume
Chromatography	A term for methods of separation based upon the partition of analyte species between a stationary phase and a mobile phase
Column Chromatography	Methods in which the stationary phase is held within or on the surface of a column
Crucible	Vessel used to hold sample. Also know as container or pan
Crystalline	Solid form
Crystallisation	Formation of crystalline substances from solutions, melt or the glassy state
Decomposition	Breakdown or change of a material or substance (by heat, chemical reaction or other process) into other chemical compound.
DSC	Instrument for measuring the differential energy supplied between a sample and reference to maintain a minimal temperature difference between the sample and reference in response to a temperature program

Endotherm	Deviation from the sample baseline of a DSC curve indicating energy absorption by the sample relative to a reference
Enthalpy	Sum of the internal energy of a system plus the product of the system volume multiplied by the ambient pressure.
Exotherm	Deviation from the sample baseline of a DSC curve indicating energy release by the sample relative to the reference
Fusion	See *Melting*
Gas Chromatography	Methods that make use of a gaseous mobile phase and a liquid (GLC) or a solid (GSC) stationary phase
Glycerol	The most common natural carrier for acyl groups and the basis of many lipid classes
Heat-flux type DSC	Commercial name for quantitative DTA
Heating rate	Rate of temperature increase in response to a temperature program
HPLC	A term for column methods in which the mobile phase is a liquid, often under pressure
Instrument baseline	DSC curve recorded in the scanning mode when there is no sample and reference present
Melting	Change of state of a substance from a solid phase to a liquid phase. Also known as fusion
Melting temperature	Temperature of transition from a solid phase to a liquid phase
Mobile phase	A liquid or a gas that carries analytes through a liquid or solid stationary phase
Mole	The SI base unit for the amount of substance
Myristic acid	Tetradecanoic acid, this alkanoic acid is present in coconut oil and palm kernel oil and as the minor component of most animal fats and fish oils

Onset temperature	Transition temperature defines as the intersection between the tangent to the maximum rising slope of a DSC peak and the extrapolated sample baseline.
Peak	General term for an endothermic or exothermic deviation from baseline.
Phase	Chemically and/or physically homogeneous region of a sample (gas, liquid, solid) with distinct boundaries which can be distinguished from other dissimilar regions of the sample
Phase diagram	Graphically representation of the phase structure of a system as a function of an experimental parameter (pressure, temperature, composition, etc.)
Phase transition enthalpy	Enthalpy change of a system due to a change of phase.
Phase transition temperature	Temperature of transition from one phase of a system to another phase.
Planar Chromatography	The term used to describe chromatographic methods that make use of a flat stationary phase; the mobile phase migrates across the surface by gravity or capillary action
Purge gas	Inert gas which replaces the atmosphere in the vicinity of a sample to standardise the experimental conditions
Qualitative analysis	An analysis in which we determine the identity of the constituents species in a sample
Quantitative analysis	An analysis in which we determine how much of a constituents species is present in a sample
Refractive index	The ratio of the velocity of electromagnetic radiation *in vacuo* and the velocity in some other medium
Spectrometric methods	Methods based on the absorption, the emission, or the fluorescence of electromagnetic radiation that is proportional to the amount of analyte in the sample

Standard reference material	High-purity material exhibiting a well-characterised phase change, which is used to calibrate a Thermal Analysis instrument.
Stationary phase	A solid or immobilised liquid upon which analyte species are partitioned; compare with mobile phase
Stearic acid	Octadecanoic acid, this C_{18} acid is the second most common saturated acid
Thermal analysis	Class of analytical methods where the nature of a sample is investigated in response to a temperature program, includes DSC, DTA, DMA, TG and TMA. Also know as thermoanalysis

CHAPTER 1

INTRODUCTION

1.1 Research background

Plants are used for many purposes including foods, treating medical ailments and for dyeing clothes. Nowadays, many researchers have focused their interest on medicinal plants due to the increasing demand for health food. Species of Zingiberaceae, Annonaceae, Araceae, Simaroubaceae and many others are known as having medicinal values [1,2]. The active compounds of the plant extracts contribute to the medicinal effects, so that a number of analyses are important in research besides the extraction techniques. All conventional and advance analytical techniques to guarantee high quality of herbal products have extensively been studied. In practice, various methods and procedures for identifying and analysing the constituents have been tested to obtain high quality of the end products. Chromatographic, spectrometric and combination of chromatographic-spectrometric analytical techniques are widely used in identifications of crude and active compounds in plant extracts [3].

This technique was applied to *Zingiber* extracts; *Zingiber officinale* Roscoe was studied in this research. Besides compound identification and characterisation, analytical methods such as GC,GC-MS, HPLC and spectroscopic methods are frequently used to determine the purity of organic compounds. A thermoanalytical

method, which makes use of differential scanning calorimetry (DSC) has been used successfully in recent years for the determination of absolute purity. This technique has been applied to large number of substances including organic and inorganic compounds, pharmaceuticals, polymers and others. In 2001, the conventional heat flux DSC was used to identify and quantify the major compounds in order to characterise the constituents in the oleoresin mixture [4].

The purity determination of organic compounds has been extensively and critically reviewed by numerous authors; Plato [5]; Plato and Glasgow [6]; Widmann and Scherrer [7]; Yamamoto *et al.* [8]; Palermo and Jen Chiu [9]; Gustin [10]; Mccullough and Waddington [11]; Sondack [12]; Flynn [13] Elder [14]; An and Sabbah [15]; Donnelly *et al.* [16]; and Giron [17]. DSC analysis in conjunction with chromatographic technique provides valuable information when determining compound purity and also aid in compound identification. Donnelly *et al.* [16] reported DSC and MS were two complementary techniques, where DSC was used to ascertain purity and MS to verify identity.

DSC is very frequently used to observe the transition behaviour. It was found as an efficient method for characterising foods. In 1985, J. Schlicter *et al.*[18] used DSC technique to investigate the extent of polymorphic transformations in pure cocoa butter and in the presence of a food emulsifier. It has been further studied by Imogen Foubert *et al.* [19] to determine the isothermal crystallization kinetics of cocoa butter to explore the transformation of the DSC crystallization peak to a sigmoid crystallization curve. In 1995, Noor Azian [20] has established optimum operating conditions of DSC to determine the specific heat capacity of triacylglycerols, the major component in palm oil, the values are important in the design of palm oil refining.

In the study by J. M. N Marikkar *et al.*[21], the use of cooling and heating thermograms from HSDSC for monitoring the presence of genuine lard (GLD), beef tallow (BT), chicken fat (CF) as adulterants in canola oil has been investigated. Mixing of animal fats especially lard and tallow in any form in food products is a cause of concern for certain religions. Canola oil samples spiked separately with BT, GLD and CF in levels were analysed by HSDSC to obtain their cooling and heating

profiles. A. F. Baroni *et al*. [22] employed DSC to examine thermal transition by monitoring peak glass transition temperatures and transition enthalpies of dehydrated tomato. Establishing the effects of scanning rate on DSC characteristics is critical to qualitative and quantitative analyses of these products. DSC is used for compound characterisation, using the temperature of melting endotherm and the associated enthalpy of fusion.

In an earlier study (1993), W. L. Kerr *et al.* [23] studied the DSC for its ability to detect thermal transition in analyzing foods such as sucrose, maltodextrin and frozen potato. After several years (2000), Kouame *et al*. [24] investigated the effect of water and glass transition on the hydrolysis of sucrose by invertase in noncrystalline carbohydrate systems. The glass transition occurs over a temperature range and it has been recognized as a possible factor affecting kinetics of enzymatic changes in low-moisture foods. The glass transition is often observed from changes in the mechanical properties as the viscous, solid-like glass is transformed to a more flowing, liquid-like state. In polymer, E. Illikova *et al*.[25] also suggest accuracies measurement procedure to get the accurate specific heat measurement on $Fe_{73}Co_{12}B_{15}$ and $Co_{67}Cr_7Fe_4Si_8B_{14}$ metallic glasses.

The present research was an attempt to apply High Sesitivity Differential Scanning Calorimetry (HSDSC) instead of using ordinary or conventional DSC for identification of the major compounds in the crude product based on their thermal behaviour. HSDSC can be used as a control or a routine tool for verifying the identity of a compound. The identity of the compound was checked qualitatively by its melting point and compound purity was determined quantitatively by HSDSC method. Amar *et al*. [26] characterise the thermodynamics of protein denaturation. β-Lactoglobulin using DSC technique and the transition was found to be irreversible due to aggregation occurring.

Before this in 1999, D. Torreggianni *et al.*[27] investigate how added carbohydrates affect the colour stability of frozen strawberry juices stored at high temperature, i.e. above T_g the glass transition temperature of the maximally freeze concentrated phase. J. Ford *et al*.[28] has shown the ability of HSDSC to measure

very low amorphous content in lactose, carbamazephine and nifedipine a glassy and amorphous nature structure in pharmaceutical material.

1.2 Zingiberaceae

The Zingiberaceae is a plant family made up of wild and cultivated species. It comprises about 1200 species of which about 1000 occur in tropical Asia. Botanically, *Zingiber* gives its name to the whole ginger family, Zingiberaceae [29]. Several Zingiberaceae plants have been reported to be useful in traditional medicines [30].

1.2.1 *Zingiber officinale* Roscoe

Ginger, the rhizome of *Zingiber Officinale* Roscoe, is one of the most popular spices. It has been used since ancient times mainly for flavouring and medicinal purposes. It has good demand in the international and even local market for preparing ginger oil, oleoresin, ginger juice and essence. Ginger rhizome contains a mixture of an essential oil, a fixed oil, pungent compounds, starch, and others, such as saccharides, proteins, cellulose, waxes, colouring matter, trace minerals and etc. Starch is believed to be the most abundant of these components comprising of 40 - 60 % w/w of the dry rhizome. Previous research found that the percentage of the crude protein was 6.2 - 19.8 %, total lipid was 5.7 - 14.5 % and crude fibre was 1.1 - 7.0 % [31]. They compared these three prominent components in different cultivars at different maturity stages of the ginger rhizomes.

Oleoresin (crude extract) is one of valued ginger product, has been obtained by solvent extraction, followed by removal solvents, while ginger oil is obtained by water extraction or steam distillation. The dried rhizome of ginger contains approximately 4 - 7.5 % oleoresin [32]. The composition of the ginger rhizome is summarised in the pie chart in **Figure 1.1.**

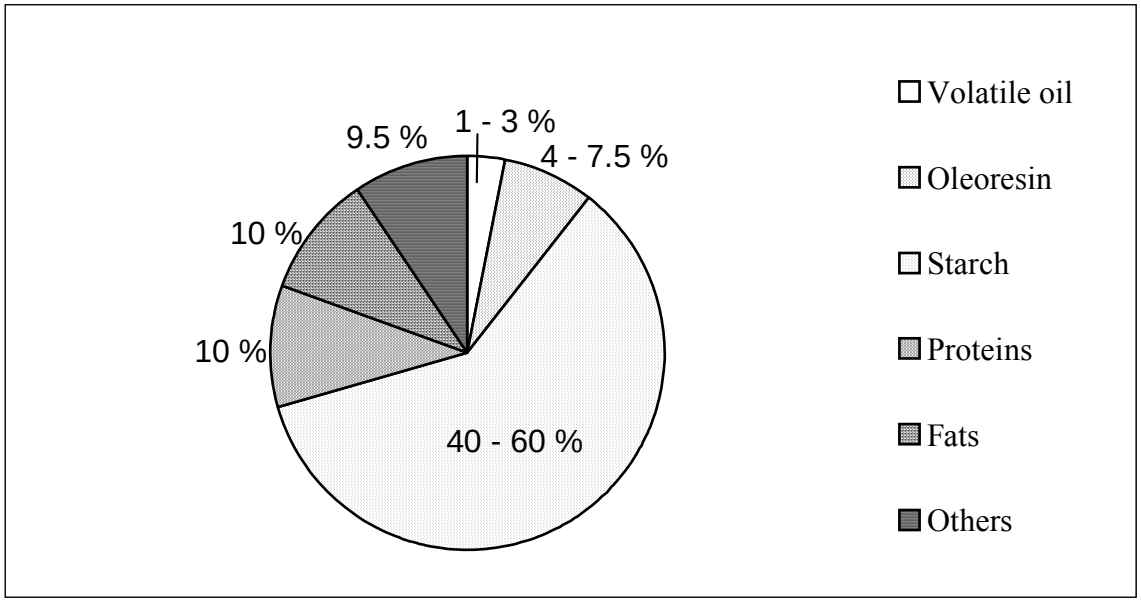
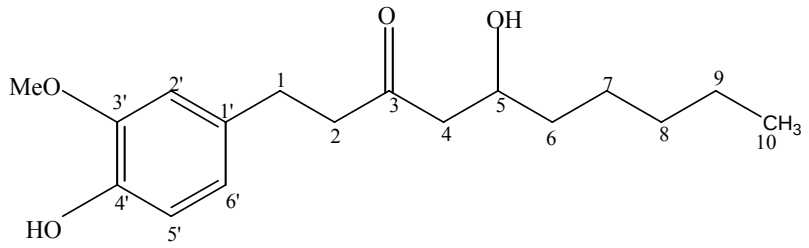


Figure 1.1: The percentage constituents of the ginger rhizome (*Zingiber officinale*) [32]

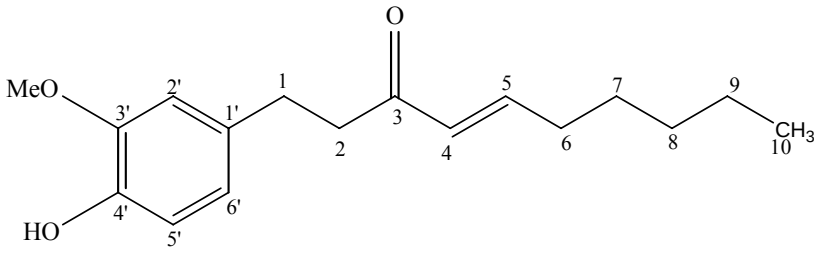
The oleoresin of ginger contains the pungent principles and non-pungent substances in addition to the essential oil content, which is about 28 % [33]. However, the quantitative composition of oleoresin is believed to vary according to the source of material, solvent used and conditions of preparation. Oleoresin or standardised ginger root extracts, known as Zinaxin™ is a registered trade name, which is commercially available in the market. The product is similar to other preparation also known as *Zingiber officinale* and gingerols.

6-gingerol (1) is a major constituent of ginger oleoresin responsible for ginger's medicinal effects. Specifically, gingerol has been identified as anti-bacterial, anti-emetic, anti-inflammatory, anti-oxidants, anti-prostaglandin, anti-rheumatic, anti-tumor, anti-ulcer and many others [34-39]. Another constituent, 6-shogaol (2) may also act as an active anti-prostaglandin compounds and anti-ulcer [40]. The non-volatile pungent principles that accounts for the characteristic aroma of ginger are gingerol, shogaols and zingerone (3). Besides that, the minor compounds are α-zingiberene (4), 6-paradol, gingerdiones, gingerdiols, linalool, camphene , citral and borneol . Compound 4 was recognised as the major

component in ginger oil [41]. The structures of the major constituents are shown in **Figure 1.2** and the physical properties of the constituents are given in **Table 1.1.**



(**1**)



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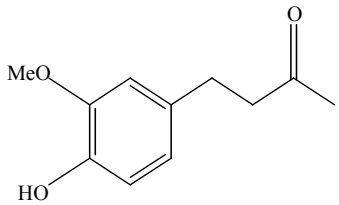

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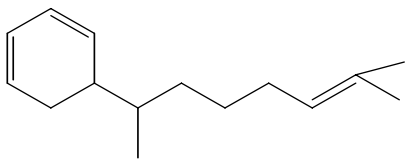


(**4**)

Figure 1.2: Structures of the prominent constituents in ginger oleoresin [42]

Table 1.1: Physical Properties from Literature for the Prominent constituents in *Zingiber officinale* Roscoe Extracts [42]

Constituents and characteristic	Molecular formula	Melting point (ºC)	Boiling point (ºC)	UV Max.	Refractive index	Mw
6-Gingerol -yellow oil -obtained as pungent -50% soluble in alcohol, ether, chloroform, benzene	$C_{17}H_{26}O_4$	29 [43]	bp_{18} 235 - 240 bp_6 227 - 229	282; 284	η_D^{20} 1.5224 η_D^{20} 1.5212	294
6-Shogaol -pale yellow oil	$C_{17}H_{24}O_3$	25.2 - 26.2 [44]	$bp_{2\text{-}2.5}$ 201.3		η_D^{25} 1.5252	276
Zingerone -sweet odour -slowly volatile in steam -constituents of ginger oil -crystals from acetone -sparingly soluble in water, PE	$C_{11}H_{14}O_3$	40 - 41	bp_{14} 187 - 188			194
Zingiberene -constituents of ginger oil -natural oil	$C_{15}H_{24}$		bp_{14} 134		η_D^{20} 1.4956	204

6-Gingerol and 6-Shogaol are the most important pungent compounds of ginger products. Other gingerols and shogaols are less important. A pungency study has indicated that 6-Gingerol is much more pungent than 6-Shogaol. 6-Shogaol and zingerone were substantially absent in ginger but only formed in the oleoresin during or after preparation [33]. The physical properties of the major constituents are given in Table 1.1. The data were limited to melting and boiling points data. Other data were not documented.

1.3 Research Objectives

The aim of this research was to study the possibility of using high sensitivity DSC in analysing natural products, especially for the family of Zingiberaceae plants, specifically melting point and heat of fusion. By doing so, a thermal analysis correlation between crude ginger oleoresin and pure active compounds will be define.

1.4 Research Scopes

In order to achieve the objectives mentioned earlier, three scopes have been identified:

1 Fresh Indonesian ginger (*Zingiber officinale* Roscoe) of grade A obtained from Pasar Selayang were used to prepare the dried ginger. A standard procedure of sample were developed and observed throughout this research work.

2 The extraction of ginger oleoresin was through solvent extraction using ethanol followed by short path distillation, rotary evaporator and microdistiller as the final step in purification.

3 Isolation and purification major compounds of the pungent principles of *Zingiber officinale* Roscoe eg gingerol, shogaol, zingerone and zingeberene compound. These standards must be first obtained in a pure form from the supplier such as Wako Pure Chemical Ind. Ltd..

4 Establish the HSDSC optimum condition (optimum scanning rate) in order to minimise errors and variations.

5 Qualitative study and design correlation of thermal behaviour; the melting point and heat of fusion data of the crude extract and pure active compounds.

CHAPTER II

HIGH SENSITIVITY DIFFERENTIAL SCANNING CALORIMETRY (HSDSC)

2.1 Introduction to Analytical Equipment

The analytical equipment that was used in this research work was high sensitivity differential scanning calorimeter (HSDSC), which is Diamond DSC from Perkin Elmer that used power compensated type of Differential Scanning Calorimeter (DSC) instead of using heat flux conventional thermal analysis. Field trips were undertaken to locate current HSDSC instrument in peninsular Malaysia. **Table 2.1** is the list of all locations and type of DSC, whether the instrument is suitable or not. The instrument only categorized suitable if it used power compensated Differential Scanning Calorimeter, which is DSC 7 or Diamond DSC. **Figure 2.1** shows an illustration of DSC development from ordinary heat flux DSC that control scan rates from 0.1 °C/min to 300 °C/min to modern and fast scan DSC that can control scanning rates between the ranges of 300 °C/min to 500 °C/min.

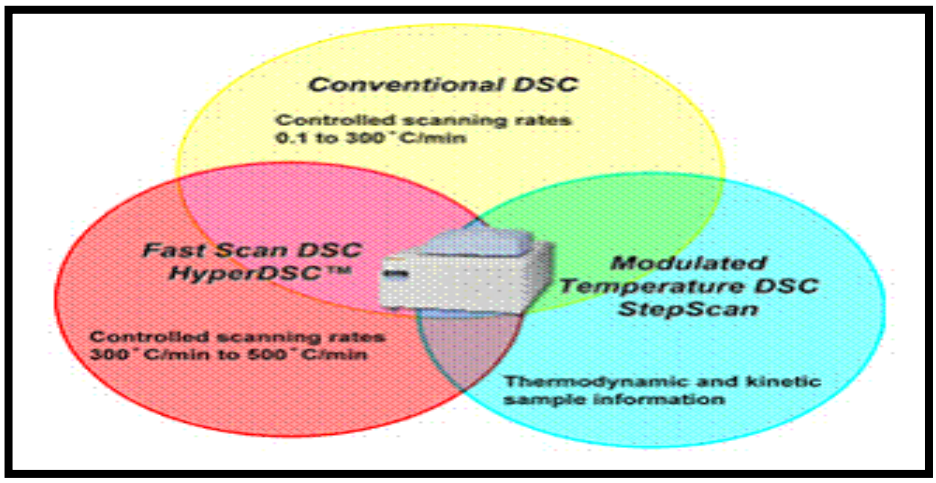


Figure 2.1 : DSC Development as Thermal Analysis [49]

Table 2.1 : Locations and Type of DSC are Available in Malaysia

LOCATION	TYPE OF DSC	STATUS
University Technology MARA (UiTM), Shah Alam	Diamond DSC Perkin Elmer	Suitable
University Technology Malaysia (UTM), Skudai	DSC 7 Perkin Elmer & DSC 821e Mettler Toledo	Not Suitable
University Putra Malaysia (UPM), Serdang	DSC 7 & Diamond DSC Perkin Elmer	Suitable
Rubber Research Institute (RRI), Sungai Buloh	DSC 822e Mettler Toledo	Not Suitable
International Islamic University of Malaysia (UIAM), Kuala Lumpur	DSC 822e Mettler Toledo	Not Suitable
Malaysian Agriculture Research and Development Institute (MARDI), Serdang	Diamond DSC Perkin Elmer	Suitable
Standards and Industrial Research Institute of Malaysia (SIRIM), Shah Alam	DSC 822e Mettler Toledo	Not Suitable

2.2 High Sensitivity Differential Scannning Calorimeter (HSDSC)

Thermal analysis in general and high sensitivity differential scanning calorimeter (HSDSC) in particular, is one of the fastest growing analytical technique which has been used for certain test like purity determination that can be performed for pharmaceuticals and organic material since development of commercial DSC instrument in the early 1960s. HSDSC has proven to be a rapid, accurate, precise and easy to use [45]. It is a breakthrough method for materials characterization, providing extra sample information in seconds rather than minutes. HSDSC can run samples as fast as 500°C/min under control, measuring the real sample temperature, whereas, for a conventional DSC method, the maximum scan rate is normally 20°C/min or 40°C/min. As a result of these fast scanning rates, throughput is at least 10 times faster and because of the conventional DSC slow heating rate, some materials may experience re-crystallization during the melting process or decompose immediately after melting. This may cause difficulty in obtaining a true analysis and may also generate some additional, unexpected thermal phenomena.[45].

In 1990, W. J. Sichina [45], has reviewed used of HSDSC for characterization of starches. For the successful characterisation of starches, it is important to have high performance DSC instrument, as the critical transitions associated with starches are very weak and can be difficult to detect. In 1996, Edward Kolbe *et al* [46] has participated also in a thermal analysis study to determine onset and peak transition temperatures and enthalpy, for both starch gelatinization and tristearin melting and crystallization using 3 types of DSC. The development of HSDSC is particularly impressive. Phil Robinson *et. al.*[47] used the HSDSC to analyze cocoa butter, one of the main ingredient used to produce chocolate, can exist in multiple and unstable crystalline forms, known as polymorphs. The given processing or thermal history of the cocoa butter and chocolate can generate different polymorphic forms which will affect the final properties (melting characteristics, appearance and physical handling) of the chocolate. The melting properties of cocoa butter and chocolate are very important, as the melting is what delivers the ‘feel’ and taste of the chocolate to the mouth. It

becomes important to characterize and understand the melting properties of the chocolate as it is related to essential consumer aspects.

In 2003, Svenja G. et al.[50]. A good quality DSC is capable of yielding satisfactory 'partial fusion' when used in stepwise and isotherm scanning mode. The term of compensated DSC is widely used by crime investigation unit to detect single fiber in forensic application. Applications of HSDSC include the characterization of polymers, fibers, films, thermosets, elastomers, composites, pharmaceuticals, foods, cosmetics, as well as organics and inorganic. HSDSC provides valuable and important information on the following important properties of materials:

· Glass transition or Tg
· Crystallization points
· Percent crystallinities
· Compositional analysis
· Purities
· Thermal stabilities
· Melting points or Tm
· Heats of fusion
· Oxidative stabilities
· Heat capacities
· Polymorphism
· Heat set temperatures

2.2.1 Principles of HSDSC

In 1995, U. Jorimann, *et al*. [51] has discovered by increasing heating rate, it will increase the sensitivity and by making right selection heating rate it will definitely give high resolution. The *Perkin Elmer Diamond* HSDSC is the differential scanning calorimeter that used power compensated principle, the sample and reference materials are each held in a separate, self-contained calorimeter, with its own heater element **(Figure 2.2).** When an exothermic (heat yielded) or endothermic (heat absorbed) change occurs in the sample, power or energy is applied to or removed from one or both of the calorimeters to compensate for the energy change occurring in the sample. The power compensated HSDSC system is maintained in a "thermal null" state at all times. The amount of power required to maintain the system in equilibrium conditions is directly proportional to the energy changes occurring in the sample. In HSDSC small furnace features two 1-gram is used while heat flux systems use on large furnace (**Figure 2.3**). This size difference

results in faster heating and cooling rates and therefore provides a true measure of the calorimetric properties of the sample since the fundamental measurement with the power compensated DSC is energy flow.

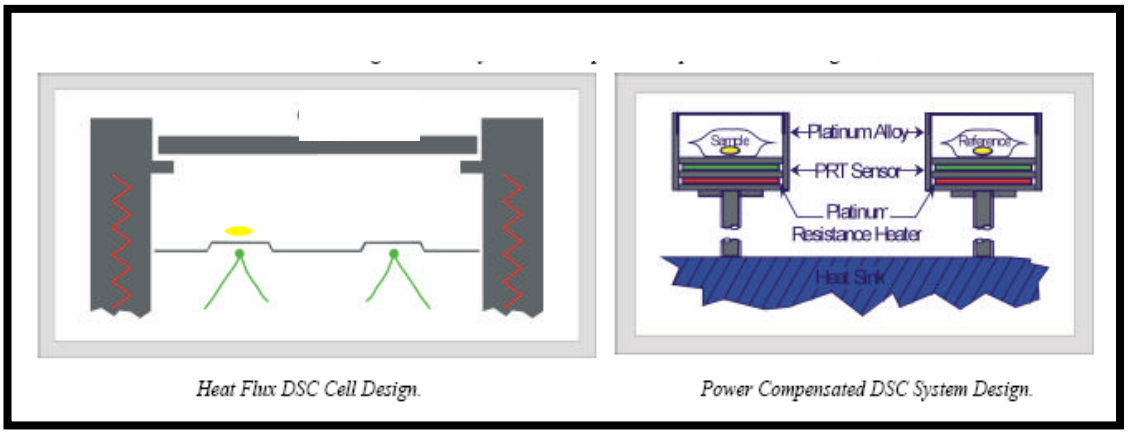


Figure 2.2: Heat Flux and Power Compensated System Design [51].

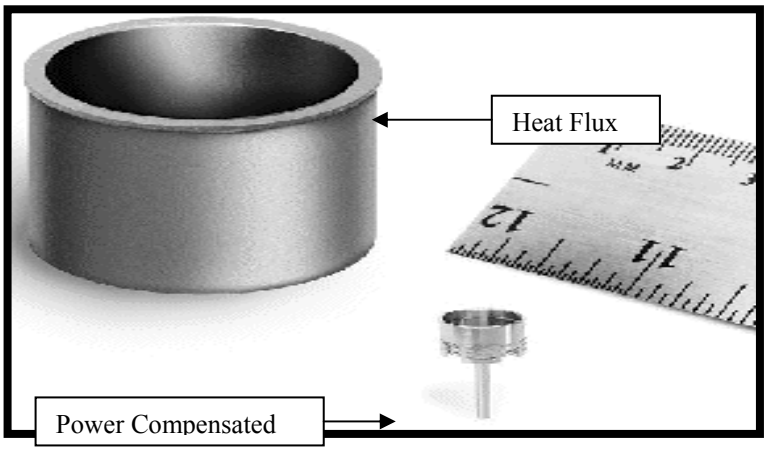


Figure 2.3: Differences in Size Furnace [51].

Paul Gabbot *et al.*[52] had proven HSDSC technique is only possible on power-compensation DSC, which measures heat flow directly and does not require complex mathematics to get premium results. Since the DSC output is mW (J/sec), HSDSC gives increased sensitivity with shorter scan times and smaller sample mass. The HSDSC analysis allows the measurement of small samples down to a few micrograms. Examples of small samples are very thin layers in multilayer films, coatings or very small amounts of materials for pharmaceutical development.

Another major interest in the pharmaceutical industry is the study of polymorphism. The use of fast scanning rates can reveal the dependence of the polymorphic transitions and can allow better interpretation of related processes.

In contrast, the more common heat flux DSC instruments have the sample and reference in a single furnace. Thermocouples measure the temperature differential (not energy differential) between the sample and reference platforms. With the heat flux DSC devices, the fundamental measurement is temperature differential rather than the thermodynamically pure energy flow. With the heat flux DSC units, the temperature differential is converted to energy flow via a mathematical equation and is a more indirect approach as compared to the pure energy flow measurements obtained via the power compensated HSDSC. The large mass of the heat flux furnace is simply not possible to heat or cool at HSDSC rates (**Figure 2.3**). The following thermogram shows the difference in performance at the rate of 100°C/min, which is the fastest rate that this heat flux instrument will achieve (**Figure 2.4**) [52].

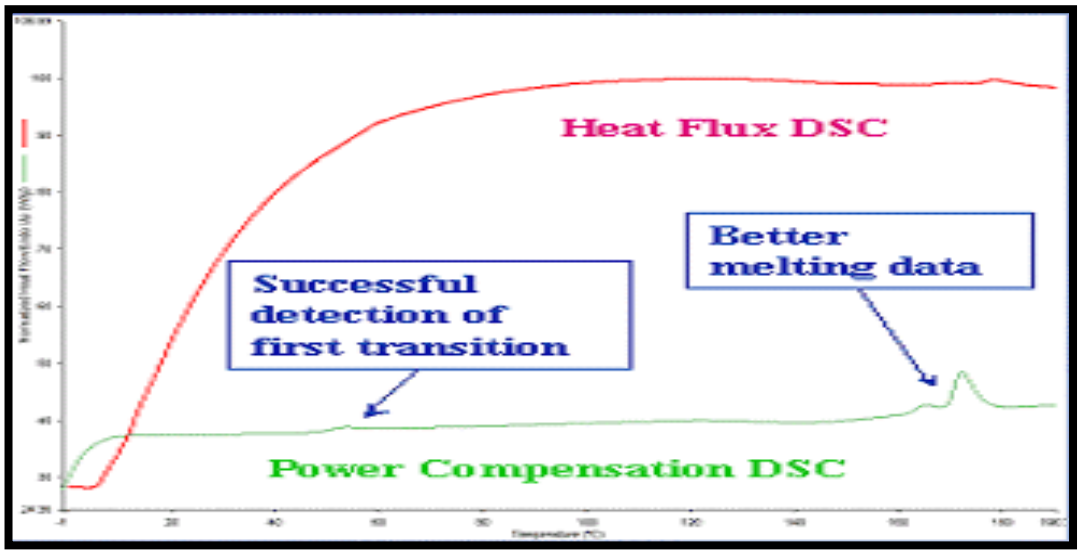


Figure 2.4 : Difference between heat flux and power compensated DSC in thermogram at the rate 100°C/min [52].

One major advantage of the power compensated design over the heat flux DSC cell is that the masses of the individual furnaces of the power compensated system are much lower than that of the heat flux. The mass of the power compensated furnace is only 1 g as compared to 100 to 200 g for most heat flux devices. The thermodynamics and physics of the low mass furnace translates into an extremely fast response times (due to less thermal inertia,) and the ability to achieve much faster heating and cooling rates as compared to the more sluggish and massive heat flux DSC furnace. The faster response time of the power compensated DSC also provides a much higher degree of resolution as compared to heat flux DSC devices. Resolution is a measure of the DSC to separate out closely occurring transitions. This is important for applications such as polymorphism of pharmaceuticals and food oils/fats, detection of heat set temperatures of polymers and fibers, characterization of liquid crystalline materials and the study of the melting properties of blends [52].

The Perkin Elmer Diamond HSDSC thermal analysis is comprised of intracooler, Hewlett Packard modified computer consist of Pentium IV and Pyris Diamond DSC software, an output device for making a permanent record and a visual display unit. **Figure 2.5** shows the block diagram of Diamond HSDSC Perkin Elmer model.

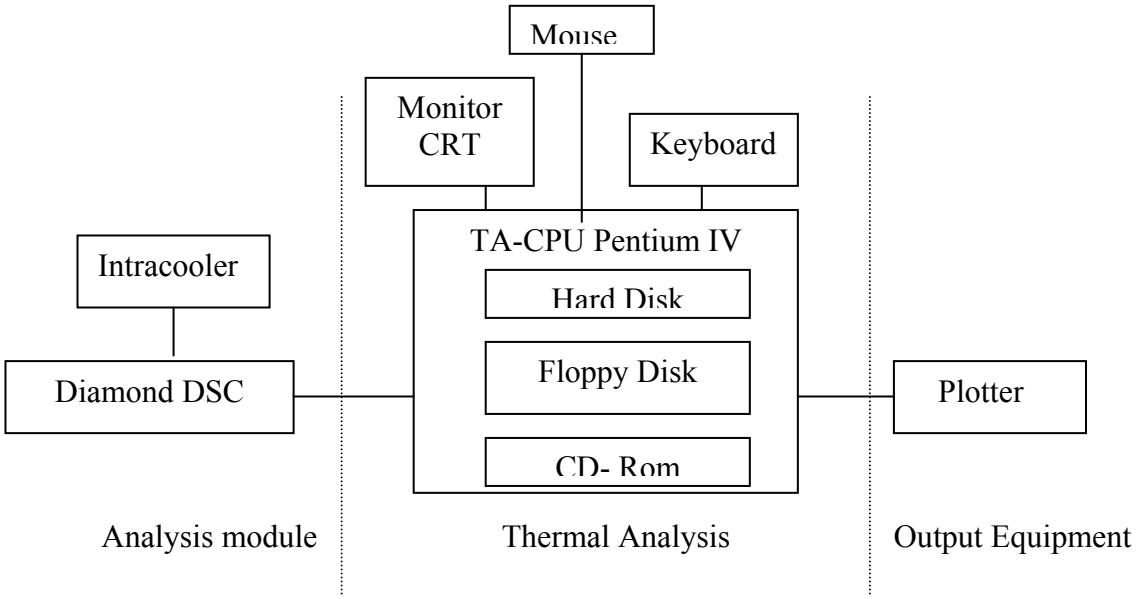


Figure 2.5 The Block Diagram of the Perkin Elmer Diamond HSDSC Equipment.

An overview of the Diamond HSDSC Perkin Elmer (**Figure2.6**) and equipment specification (**Table 2.2**)

Figure 2.6 : Diamond HSDSC Perkin Elmer, MARDI Serdang.

Table 2.2 : Specification of Diamond HSDSC Perkin Elmer

Specifications	Description
DSC Type	Power-compensation temperature null principle. Measures temperature and energy directly, rather than differential temperature (DT).
DSC Cell	Independent dual furnaces constructed of platinum-iridium alloy with independent platinum resistance heaters and temperature sensors with furnace mass less than 1g.
Temperature Sensors **Atmosphere**	Distributed, Platinum Resistance Thermometers for best linearity. Static or dynamic, including nitrogen, argon, helium, carbon dioxide, air, oxygen or other inert or active gases, over full temperature range. Oxygen can be used up to 730 °C which allows easy cleaning.
Temperature Range	Range -170 °C to 730 °C
Calorimetry	Accuracy / Precision < ±1% / < ±0.1% Sensitivity 0.2 μW Dynamic Range 0.2 μW to 800 mW
Signal Response	(1 mg Indium, 10 °C/min, nitrogen purge) Peak Height 7.44 mW ± 0.15 mW Width at half height 0.42 ± 0.10 °C H/W Ratio 17.6 mW/°C ± 1 mW/°C
Isothermal Drift (10 min)	-150 °C / 100 °C < 15 μW / < 10 μW
Scanning Rates	Heating/Cooling 0.01 °C to 500 °C/min
Temperature Overshoot	100 °C/min < 0.1 °C
Cooling Options	Ice Water 25 °C to 730 °C Circulating Liquid -10 °C to 730 °C Refrigerator (Intracooler) -70 °C to 730 °C Automatic Liquid N2 (CryoFill) -170 °C to 300 °C

2.2.2 Definitions and Terms

The temperature of any process in the sample must be determined from the measured curves. **Figure 2.7** shows the terms used for the description of the measured curves. The explanation is given below [53, 54]:

Measured curve: Trace of the measured signal as a function of time or temperature (furnace temperature or sample-sensor temperature).

Peak: Curve measured during liberation of a heat due to a transition in the sample.

Peak start, T_i: Initial deviation of the measured signal from the baseline in the region of the peak.

Peak maximum, T_p: Maximum difference between interpolated baseline and peak.

Peak end, T_f: Final deviation of the measured signal from the baseline in the region of the peak.

Melting temperature, T_m: The extrapolated onset temperature is defined as the intersection between the tangential lines through the descending slope of the peak and the linearly extrapolated initial baseline. It is also known as transition temperature, T_t as general.

Heat of fusion, ΔH_f: The area of the peak marked of by the straight line between two points (start and end temperature).

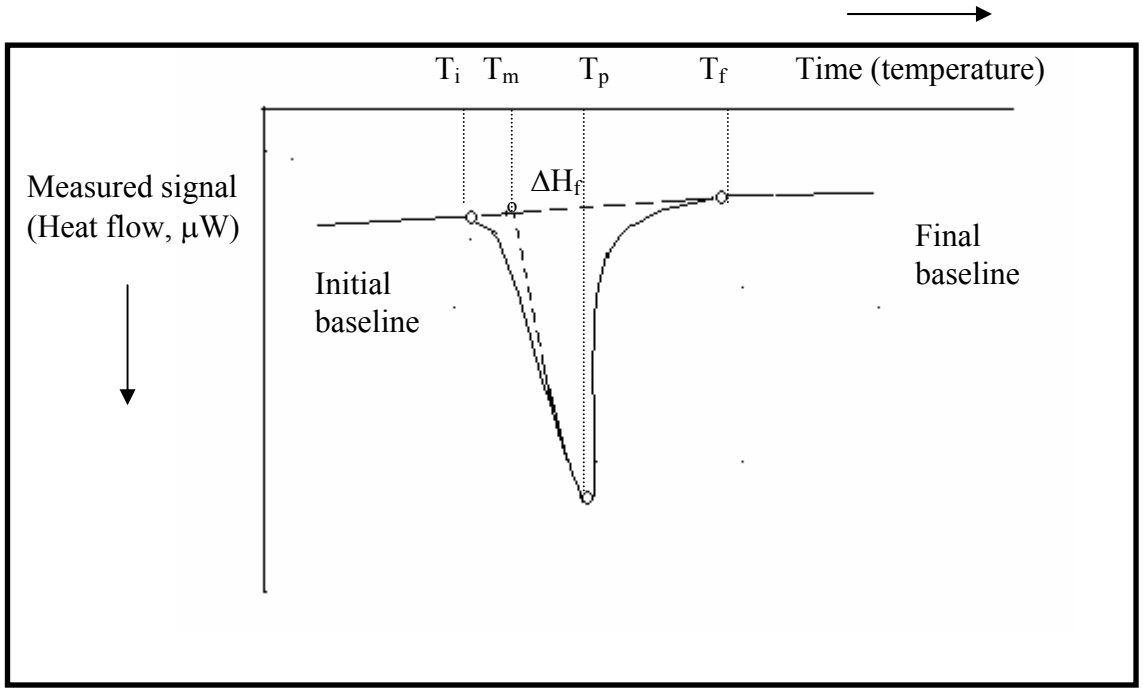


Figure 2.7: Definition of Characteristic Temperatures of a Peak [53].

2.3 Interpretation of DSC Curves

In general, each substance gives a DSC curve in which the number of peaks, shape and position of the various endothermic and exothermic features serve as a means of qualitative identification of the substance [55]. **Figure 2.8** shows a typical heat-flux DSC scan. When an endothermic change occurs, the sample temperature lags behind the reference temperature because of the heat in the sample. The initiation point of a phase change or chemical reaction is the point at which the curve first deviates from the baseline.

When the transition is complete, thermal diffusion brings the sample back to equilibrium quickly. Broader endothermic curve (T_1) cover behaviour ranging from dehydration and temperature-dependent phase behaviours of partially crystalline compounds or mixture while sharp endothermic curve (T_2) is indicative of crystalline rearrangements, fusions, or solid-state transitions for relatively pure materials. A crystallisation curve, T_3 shows positive deviation (exothermic) indicates heat evolution.

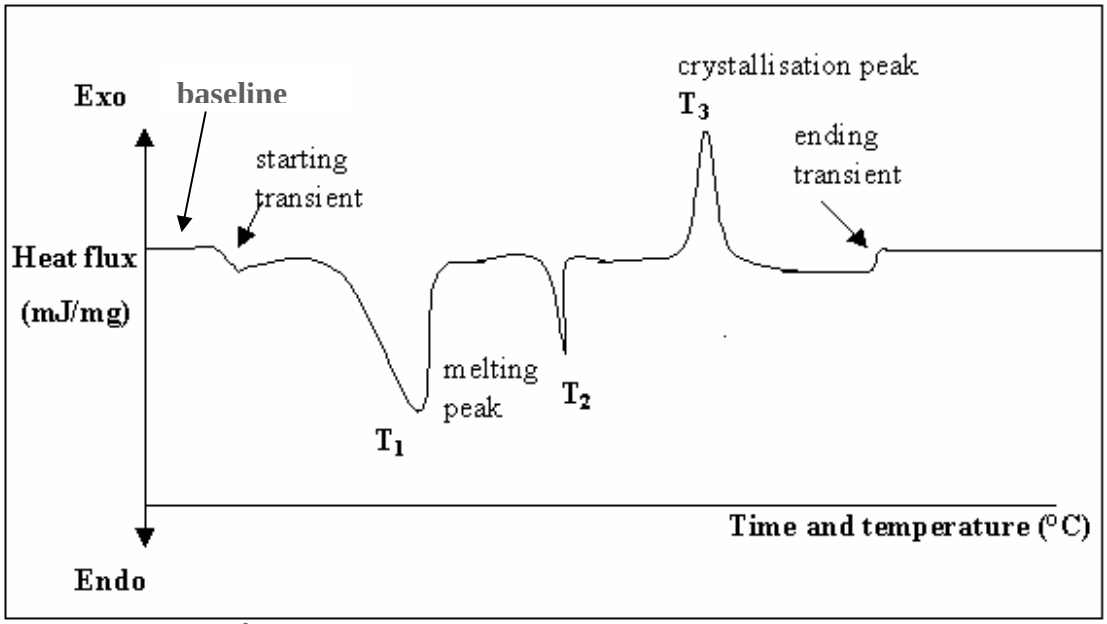


Figure 2.8 : Typical DSC Scan [55]

The heat of fusion and melting point can be determined from the melting curve. For phase transitions the calorimeter plots are represented normally by a 'peak', which is shown in **Figure 2.9.** The characteristic temperature of a peak can be determined either through onset temperature or peak maximum temperature. **Figure 2.9 (a –f)** illustrate the possible reactions when samples are heated. With pure substances, where the low temperature side of the melting peak is almost a straight line (**Figure 2.9 a**), the melting point corresponds to the onset temperature. Impure and mixture sample often show several peaks, whose melting curve are concave in shape, are characterised by the temperature of their peak maximum [56] (**Figure 2.9 b and c**).

Since the peak maximum temperature depends heavily on sample mass and shape while the onset temperature is independent of sample parameters [54], thus only the extrapolated onset temperature should be taken for melting point determination. Substance with eutectic impurities exhibit two peaks (**Figure 2.9 b**), first the eutectic peak, whose size is proportional to the amount of impurity and then the main peak. Sometimes the eutectic is amorphous so the first peak is missing (**Figure 2.9 c**). Partially crystalline substances give rise to very broad melting peaks because of the size distribution of the crystallites. Many organic compounds melt

with decomposition (exothermic or endothermic). The curve can be clearly seen in **Figure 2.9 (d)** and **2.9 (e)** [56].

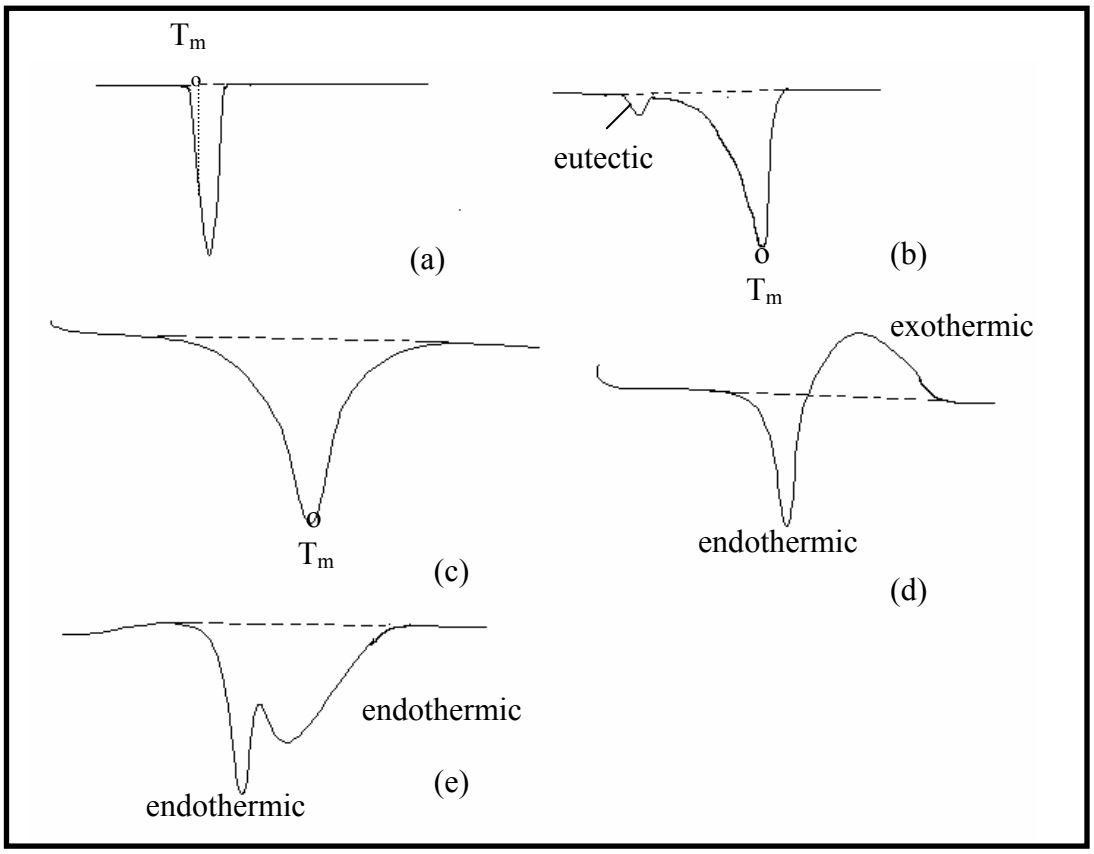


Figure 2.9: Melting Processes: (a): a Pure Substance; (b): a Sample with a Eutectic Impurity; (c): a Mixture; (d) and (e): Melting with Decomposition [56]

2.4 Purity Determination Using DSC

Measurements of the depression of the melting point of a sample are often used to determine its purity [57]. Calculations are based on the assumption that solid solutions are not formed and that the melt is an ideal solution. The melting endotherm for a pure substance recorded on a DSC is illustrated in **Figure 2.10 (a).** T_0 is the melting point of the sample and the area ABC is proportional to the enthalpy of fusion, ΔH_f, of the sample. Compare with the presence of impurity in the sample generally lowers the melting point and also broadens the melting range, giving a broader DSC endotherm as illustrated in **Figure 2.10 (b).**

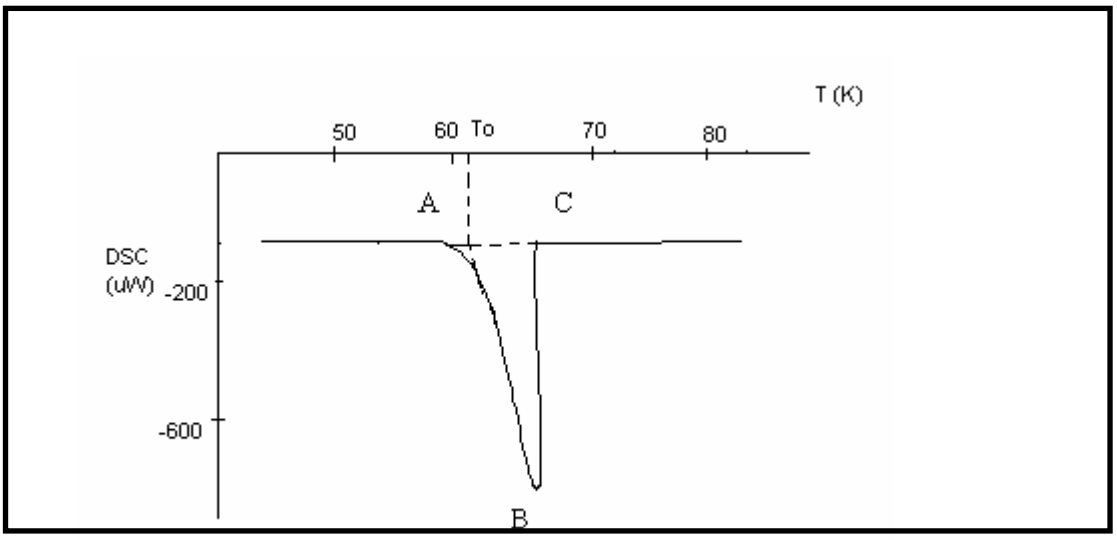


Figure 2.10 (a): Idealised DSC Record of Melting of Pure Sample. T_0 is the Melting Point of a Theoretical Sample with Zero Impurity. Area ABC Represents the Enthalpy of Fusion, ΔH_f. [57]

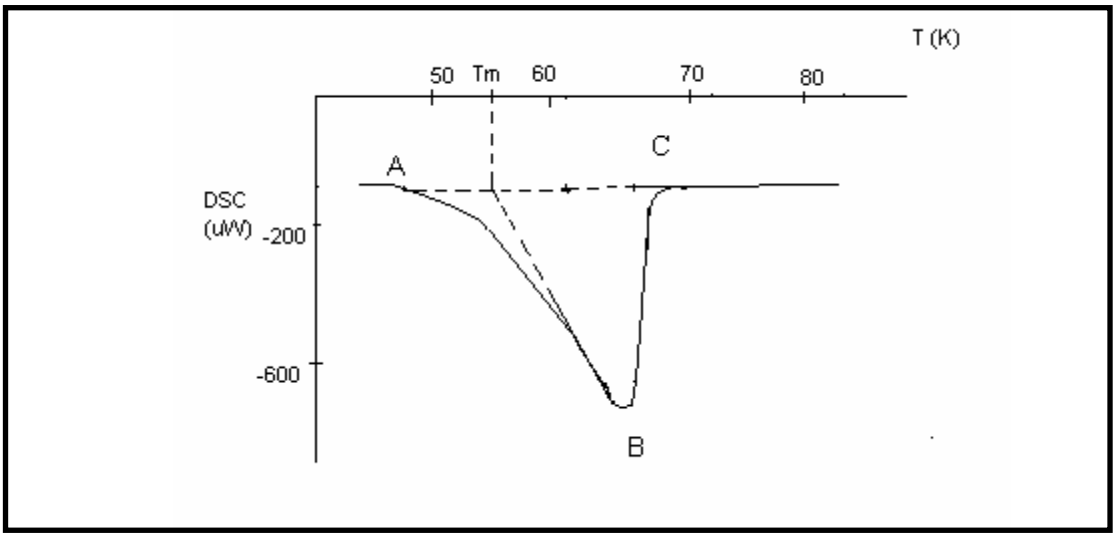


Figure 2.10 (b): Idealised DSC Record of Melting of an Impure Sample. T_m is the Melting Point of a Sample. The Area ABC Represents the Enthalpy of Fusion, $\Delta H_{f,A.}$ [57]

2.5 Phase Diagram of a Pure Substance

Equilibrium can exist not only between the liquid and vapour phases of a substance but also between the solid and liquid phases, and the solid and gas phases of a substance. A phase diagram is a graphical way to depict the effects of pressure and temperature on the phase of a substance [58]. **Figure 2.12** illustrates a phase diagram of a pure substance.

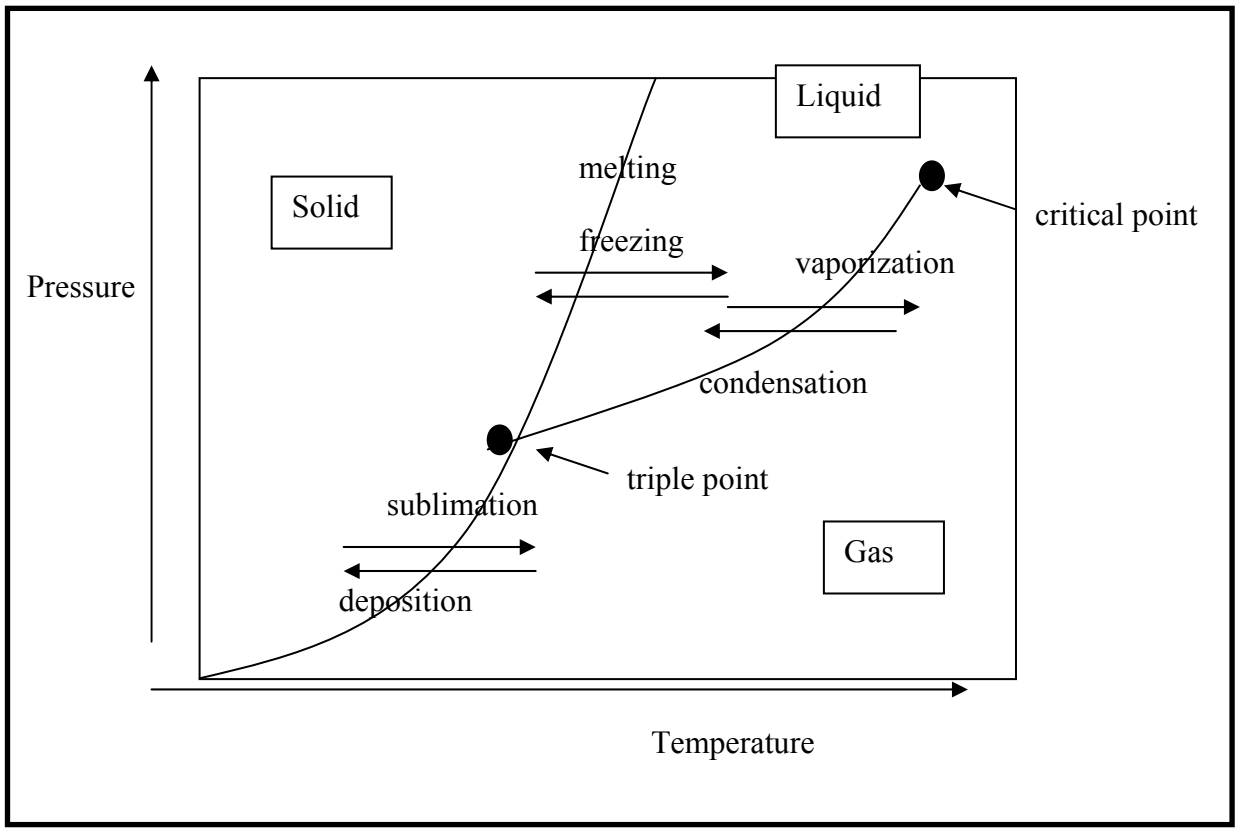


Figure 2.12: A Phase Diagram of a Pure Substance [58]

The curves indicate the conditions of temperature and the pressure under which equilibrium between different phases of a substance can exist. The vapour pressure curve is the border between the liquid and gaseous states of the substance. The vapour pressure curve ends at the critical point. The line between the gas and solid phase indicates the vapour pressure of the solid as it sublimes at different temperatures. The line between the solid and liquid phases indicates the melting temperature of a solid as a function of pressure. The triple point is the particular

condition of temperature and pressure where all three physical states are in equilibrium. Regions not on a line represent conditions of temperature and pressure where only one particular phase is present.

CHAPTER III

METHODOLOGY

3.1 Overall Research

Methodology for the whole research would be referring to the overall flowchart Figure 3.1. Basically, there are 5 main parts in the research:

1) Preliminary survey to locate HSDSC, the Diamond DSC from Perkin Elmer in peninsular Malaysia, conducted through out visits.
2) Extraction of pure ginger oleoresin compounds using solvent extractor
3) Isolation of pure ginger oleoresin compounds using gravity liquid chromatography
4) Elucidation and confirmation of the pure structures using Thin Layer Chromatography and comparing with the standard.
5) Qualitative and quantitative analysis development using HSDSC.

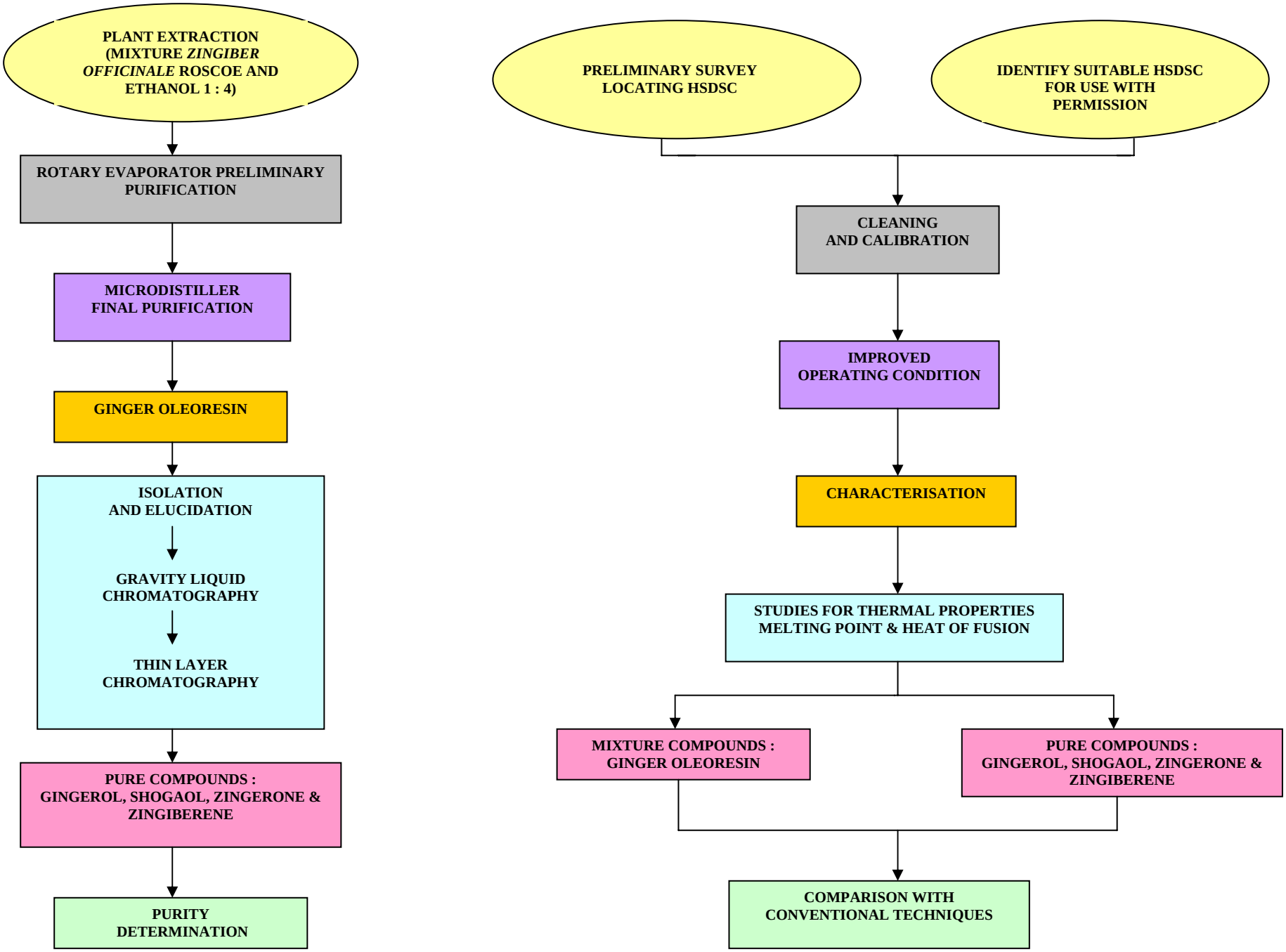


Figure 3.1: Overall Methodology

3.2 General Procedure

Mature ginger rhizomes (*Zingiber officinale* Roscoe) were obtained from a supplier in Pasar Borong Selayang, Selangor. The major compound in ginger oleoresin, 6-gingerol standard was purchased from Wako Pure Chemical Industries (Osaka, Japan). All reagents (chloroform, petroleum ether, diethyl ether and ethyl acetate) were of analytical grade (LabScan Asia, Co.) and were used without further purification except for petroleum ether (LabScan Asia, Co.) and glycerol (Fisher Scientific). Two techniques were performed in this research, where dried rhizomes were extracted using solvent extraction to produce crude products (oleoresin).

3.3 Extraction of *Zingiber officinale* Roscoe

The rhizomes were washed, sliced, bleached and solar-dried. To ensure consistent oleoresin sample after solvent removal, the Refractive Index (RI) was measured using ATAGO's Abbe Refractometer 1T, accurate to ± 0.0002 and afterward sample was taken only if η_D^{20} 1.5200 [59]. In this research synthetic ethanol (Labscan Asia Co. 98% pure) were used to extract oleoresin.

The ginger rhizomes (200 g) were used and the ratio of ginger to ethanol was 1:4. The extraction process was performed in the 15 L capacity vessel extractor. The extraction time was carried out for 6 hours at 65°C and 1 atm. After this period, 98% solvent was removed under vacuum at 175 mbar in the Buchi R205 rotary evaporator. The crude extract was used as a mixture in DSC analysis.

3.3.1 Isolation of 6-Gingerol

The major compounds were separated and purified using gravity liquid chromatography (GLC). The absorbent used for column chromatography were silica gel Merck 60 (230-400 mesh) and silica gel Merck 60 (70-230 mesh). A series of solvent system with increasing polarity.

Rhizomes *Z. officinale* Roscoe (2000.00 g) were extracted using large vessel extractor in ethanol (8L) for 6 hours. The solvent was removed using Buchi R-205 rotary evaporator at constant vacuum 175 mbar, a brown viscous liquid ginger oleoresin (2.00 g) was subjected to gravity column chromatography over 10 g silica gel Merck 60 (70 - 230 mesh) in a slurry with the increasing polarity of mobile phase starting with non polar, petroleum ether to a mixture of petroleum ether and diethyl ether, followed by 100 % of diethyl ether until PE:Et_2O (4:6 v/v). Petroleum ether (60-80°C) was distilled before it can be used as eluent (mobile phase) in separation process. The slurry mixture was poured into the vertically oriented glass column (30 cm x 1.5 cm i.d.) to yield 41 fractions. The earlier fractions contained very volatile and least polar compounds present in the extracted oleoresin, followed by polar compounds and the most polar compounds. Each fraction was analysed by TLC and was performed using TLC aluminium plate, gel silica Merck 60 F_{254} with 0.20 mm thickness. The spots of the isolated compounds on TLC plate were examined under a UV lamp (254) nm and were clearly visualised by iodine vapour that form a brown spots.

Isolation and purification techniques are necessary to obtain the major compounds in the pure form in order to provide the sample for DSC investigation, which require the thermal reference data of the major compounds. The major compounds were separated and purified by means of several chromatographic techniques including vacuum liquid chromatography (VLC), gravity liquid chromatography (GLC) and preparative thin layer chromatography (TLC). The structures were elucidated using spectroscopic methods including ^{1}H NMR, ^{13}C NMR, IR and GC-MS.

3.3.1.1 Vacuum Liquid Chromatography (VLC)

The chromatographic column was prepared by mixing the adsorbent (silica gel 9385, 230-400 mesh) in slurry with the mobile phase (series of solvent mixture, starting with non polar, petroleum ether to a mixture of petroleum ether: diethyl ether with increasing polarity). The round filter paper was placed in the column, served to give flat base to the column of adsorbent and the sample while solvent was poured

and suck dry. The earlier fraction contained the very volatile and least polar compounds present in the extracted oleoresin. The following fraction contained polar compounds and next was the most polar compounds. About 25 fractions were produced from this method and the same R_f value from TLC analyses was combined to get the main fraction (6 fractions). Next step, the fraction that contains the major compounds is selected for further purification using gravity liquid chromatography (GLC). The purification procedures were simplified as shown in figure 3.7 (a). A part of the procedure has been taken a photo (Figure 3.7 (b)).

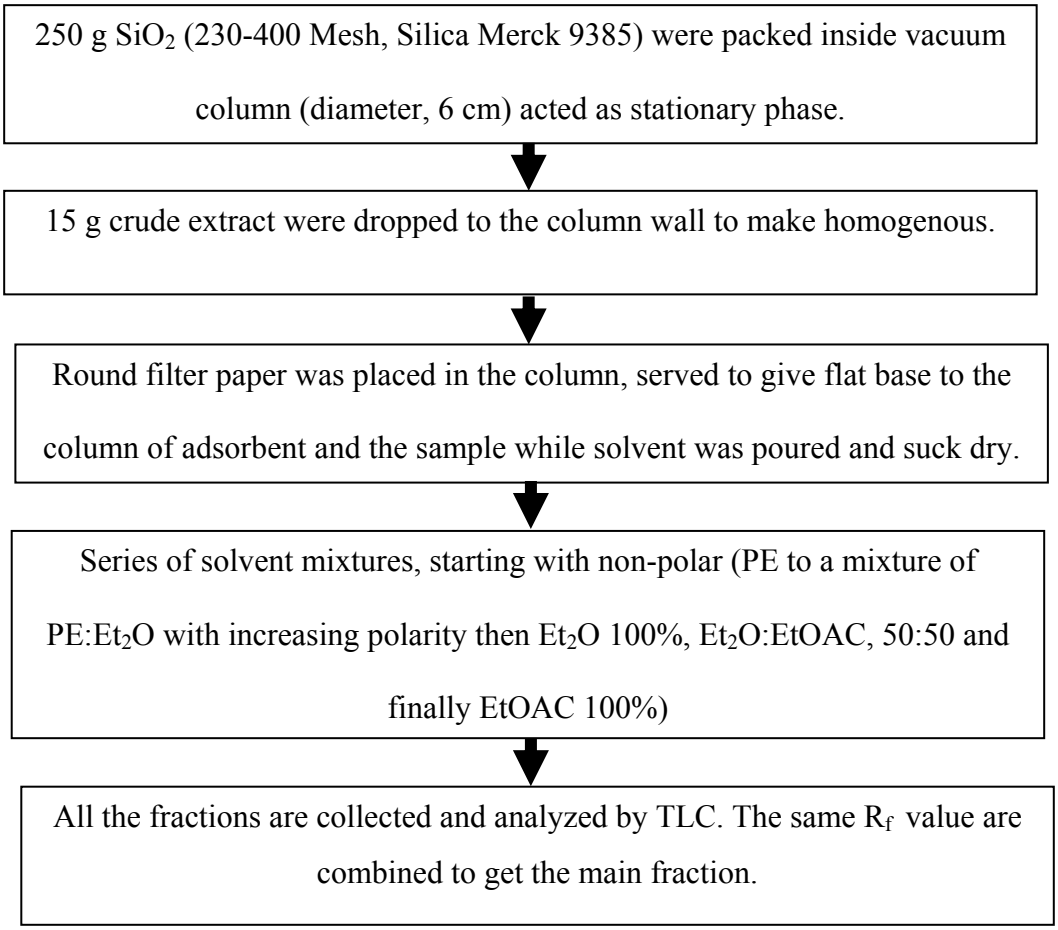


Figure 3.7 (a): The VLC procedure

(a)

(b)

(c)

(d)

Figure 3.7 (b):

(a) Sample is put homogeneously from the column wall.

(b) Sample isolated through the SiO_2 packed by the series of solvent system.

(c) Most of the sample isolated completely with 100% EtOAC (most polar).

(d) The 25 fractions are produced which every fraction will be analyzed by TLC and the same spot have to combine.

3.3.1.2 Gravity Liquid Chromatography (GLC)

The stationary phase used was (silica gel 7734) and the mobile phase (pet. ether: diethyl ether, 3:7 v/v). The adsorbent was packed in the column (30 cm x 2.5 cm i.d.) with petroleum ether into the vertically oriented glass column. The column was taped during packing to minimized air bubbles in the packing procedure. The fraction sample from GLC is placed in the column approximately 1 cm from the

adsorbent base. It must be cautioned that the level of the mobile phase must never be allowed to fall below the level of the adsorbent. This may result in the development of cracks in the adsorbent column and hence poor resolution [2]. Elution of the sample was carried out using 3:7 v/v (pet. ether: diethyl ether) by gravity action. Figure 3.7 (c) shows the GLC procedure, which is the continuous technique after the sample has been fractionated using VLC.

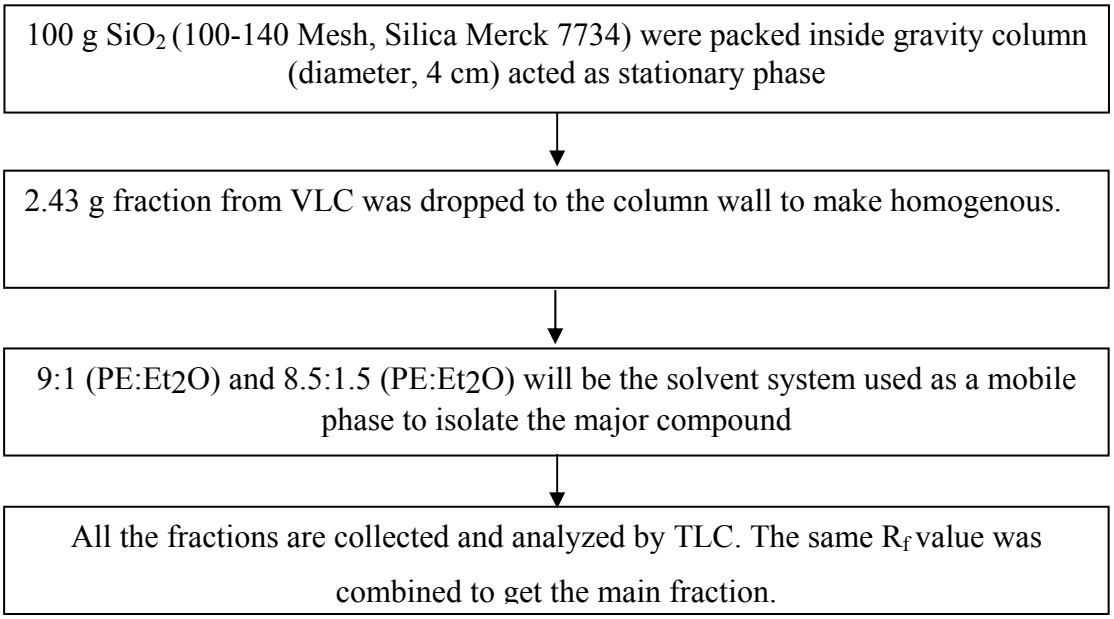
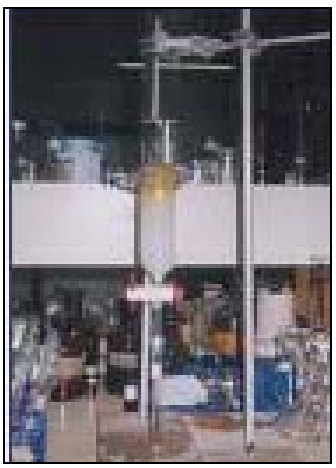


Figure 3.7 (c): The GLC procedure

Figure 3.7 (d) shows the two pictorial procedures of sample fractionation using GLC.

(a)



(b)

Figure 3.7 (d):

(a) Shogaol (the second fraction from VLC) is placed on SiO_2 packing.
(b) The sample moves through SiO_2 packing after being fractionated with the mobile phase by gravity action.

3.3.1.3 Thin Layer Chromatography (TLC)

Figure 3.7 (e) shows the steps involved in the thin layer chromatography technique.

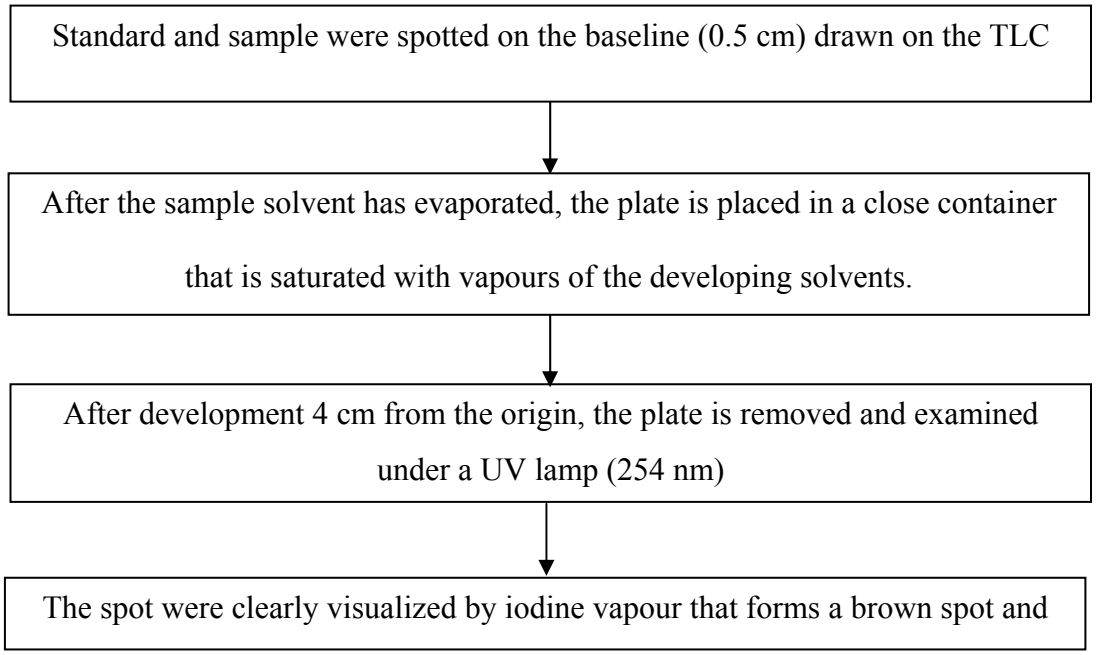


Figure 3.7 (e): The TLC procedure

^{1}H-NMR and carbon ^{13}C-NMR spectra were recorded using spectrometer NMR Varian Unity INOVA 400, which operated at 400 MHz and 100 MHz, respectively. $CDCl_3$ was used as a solvent and TMS as an internal standard. Infrared spectra of the pure components were recorded in the region of 4000-400 cm^{-1} using Fourier Transform Infrared (FTIR) Perkin Elmer Spectrometer. About 1 mg of solid sample was finely ground with about 100 mg of dry KBr. The mixture was placed in a steel die and subjected to a pressure of approximately 15,000 psi. The KBr disc was then placed in the radiation beam. In the case of liquids, a few drops of sample were placed on a flat, polished disc of crystalline NaCl and a second such disc was placed

on top. The two discs are squeezed together until an evenly distributed liquid film was obtained between them. This combination was placed into a suitable holder that fits in the radiation beam. Mass spectra (MS) were recorded using Hewlett Packard Model 5989A Spectrometer.

A Shimadzu GC 14A Chromatograph equipped with a FID detector using fused-silica CBP1 column (25 m x 0.22 mm i.d.) was employed. The samples were injected in the split mode, using pressure controlled by helium as a carrier gas at 50 cm^3/min. The injector and detector temperatures were maintained at 250 ºC. The oven temperature program from 60 ºC (10 min) to 250 ºC at 3 ºC/min and the final time was 1 min. GC-MS was recorded using Hewlet Packard Model 5890 Series II Spectometer. The analyses were carried out on a CBP1 column (25 m x 0.22 mm i.d.). The oven temperature program was 60 ºC (10 min), then 3 ºC/min to 250 ºC (1 min) with helium as carrier gas.

The main emphasis of isolation technique in this research was to isolate the pure major compounds. The yield of the isolated compound was not taken into consideration. Compound 6-Gingerol standard with 98 % purity determined by HPLC was imported from Japan and used as a standard.

3.4 General experimental procedure using HSDSC

HSDSC needs to be cleaned and calibrated before any measurement was made. The HSDSC furnace was heated until equilibrium is achieved at 50 °C. Sealed aluminium pan were used to study all the thermal properties that might be involved. A 10 mg sample mass was weighed into the pan on a microbalance and crimped. An empty pan treated in the same way was used as reference. The reference pan was placed on a reference holder, while the sample pan was placed on the sample holder in the furnace.

Generally, the HSDSC was set to a temperature programme where start temperature was -20 ºC below the melting point and end temperatures were 100 ºC above the melting point. The initial and final temperature was held for 3 minutes. All the samples were heated continuously. At least three sets of runs were carried out for every sample. The scanning was started when the baseline has been stabilised (equilibrium). Gas purging is required throughout the experimental runs for faster cooling.

3.4.1 Standard materials

The DSC calibration was performed using the following organic compounds, to check the accuracy of the instrument:

i) Glycerol with a purity of 99.8% determined by GC (Fisher Scientific)
ii) Gingerol Standard with a purity of 98.0% determined by HPLC (Wako Pure Chemical Ind. Ltd.)

The organic samples were chosen based on their thermal properties. Ideally, the thermal properties of the organic samples including its melting temperature should be as close as possible to those of the sample under consideration [60].

3.4.2 Instrumentation

DSC: The DSC heat flow measurements will be determined with power compensated Diamond HSDSC system from Perkin Elmer, at Malaysian Agriculture Research and Development Institute (MARDI), Serdang. This analytical instrument comprises a Diamond furnace, intra-cooler, a data-processing unit TA station, an output device for recording the hard copy and a visual display unit. See **Figure 3.2.** The unit comes together with the software called *Pyris* for heat of reaction and transition temperature calculation. The crucible type used was an aluminium pan.

Microbalance: The samples were weighed using Mettler Toledo MonoBLoc AB204-S, maximum and minimum weight is 220 g and 0.1mg in accuracy to ± 0.05 mg. For each experimental run, two aluminium pans were used (sample and reference) and these two pans were within ± 0.05 mg.

Figure 3.2: HSDSC Measurement at MARDI, Serdang

3.5 HSDSC maintenance

3.5.1 Cleaning

The HSDSC was cleaned every time after measurement. Cleaning the sample holder is important to check any abnormal peak before measurement has been made. Commonly the sample holder sensor and the furnace may be contaminated due to decomposition, evaporation or sublimation of the sample. Cleaning inside the furnace using acetone was performed by purging the inert gas, N_2 (30 mL/min) and raised the furnace temperature from 0 ºC to a high temperature 600 ºC to remove any impurities or volatile component that are formed from previous measurements.

3.5.2 Instrument calibration

To get precise data, HSDSC instrument must be calibrated according to the procedures described by the manufacturer in the operations manual or using ASTM methods in designation E 968[61] and E 967[62] for calorimetric sensitivity from time to time. There are two kinds of calibration, temperature calibration and heat flow calibration (E 1269)[63]. In this experiment, HSDSC was calibrated frequently (after every 10 measurements) using organic standard materials liked 6-Gingerol and glycerol. It is obvious that a good agreement with the literature values and standard material must be obtain when measuring the transition temperature.

3.5.2.1 Temperature Calibration Methods

Procedures for temperature calibration are divided by two, such as two point calibration and one point calibration that was used for substances that melted transition of >99.99% pure materials(ASTM E 967)[62].

i) Two Point Calibration Procedure

Select two calibration standard materials with melting temperatures one above and one below the temperature range of interest. The calibration materials should be as close to the temperature range of interest as

practical. Calibration materials about 5 to 15 mg were placed in a clean specimen holder. Then the specimens were loaded into the instrument chamber (furnace) with dry nitrogen being purge throughout the experiment.

Finally, calibrations materials were rapidly heated or cooled below the calibration temperatures of interest to allow it to stabilize. This calculation enables the coefficient of the thermal curve that has been recorded. If practical, adjustment to the temperature scale of the instrument should be made so that temperatures are accurately indicated directly.

ii) One Point Calibration Procedure

This procedure can only be used if the slope value previously from the two point calibrations was determined to be sufficiently close to 1.0000. All procedure is the same as two point calibrations except for using only one standard material.

3.5.2.2 Heat Flow Calibration Methods

For heat flow calibration, the two main procedures are calibration at a specific temperature and other temperatures that are used to calibrate the heat flow response of the instrument with the same type specimen holder, heating rate, purge gas and purge gas flow rate (E968)[61].

i) Calibration at a Specific Temperature

Melt transition calibration material 5 to 15 mg weighed amount was placed into a clean specimen holder and sealed with lid to minimize the free space. Then the specimen was loaded into the instrument to equilibrate at a temperature below the melting temperature.
Next the specimen was heated up until the baseline re-established above the melting endotherm and the accompanying thermal curve of

heat been recorded. The specimen was then cooled and reweighed. The data will be rejected if mass losses exceeded 1% of the original mass or if there is any reaction with the specimen holder. Finally, the coefficient of the thermal curve that has been recorded was calculated.

ii) Calibration at Other Temperature

This procedure is an interpolative technique that can be accomplished after data from calibration at a specific temperature procedure were obtained. The scan starts at least 30°C below the lowest temperature of interest (to permit attainment of dynamic equilibrium) to 10°C above the highest temperature of interest and include the temperature from above method (5.1.2.i) must be selected. Then, the sapphire was placed into the specimen holder with lid and loaded into the furnace. The initial temperature was held for 5 minutes. The specimen was heated up and held for 2 minutes at the end of the scan. The specimen was cooled and the calibration coefficient at the temperature of interest within the temperature range was calculated.

3.5.2.3 Sample Temperature Calibration

The sample temperature was corrected by measuring the melting point of indium and tin. The constants a and b were calculated from the measure melting point (x) and accepted melting point, literature (y) using the formula below:

Value to be input as T. offset

$$a = \frac{y_{tn} - y_{in}}{x_{tn} - x_{in}}\left(a' - x_{in}\right) + y_{in} \qquad ...3.1$$

Value to be input as T. gain

$$b = \frac{y_{tn} - y_{in}}{x_{tn} - x_{in}}\left(b'\right) \qquad ...3.2$$

where;

a’ = T. offset value used in the measurement = 0 initial value

b’ = T. gain value used in the measurement = 1 initial value

x_{in} = measured melting point of indium (ºC)

x_{tn} = measured melting point of tin (ºC)

y_{in} = accepted melting point of indium (156.6 ºC)

y_{tn} = accepted melting point of tin (232.0 ºC)

3.5.2.4 HSDSC Sensitivity Calibration

(Calibrating the Sensitivity Using Multiple Standards)

A second-order equation was used to find DSC span, 1 dimcal and 2 dimcal to calibrate the sensitivity using multiple standard samples when the measurement covers a wide temperature range and quantitative heat measurements were necessary. The metal used must be at least 99.999% pure and has an undisputed melting point (Indium, Tin and Zinc). DSC span, 1dim cal and 2dim cal were found using the second order equation;

$$Ki = \frac{\Delta H ref.i}{\Delta H cal.i}\left(c^{'}\right) \qquad \ldots 3.3$$

where;

Ki = equipment constant at transition temperature of substances i (uW)

ΔHref.i = accepted ΔH value for substance i (mJ/mg)

ΔHcal.i = calculated value of ΔH for substance i (mJ/mg)

c’ = DSC span value at time of measurements (uW) = initial value, 2.000E+05

The coefficients of second order regression of temperature were found with the equation below using the sets of transition temperature, Ti and equipment constant, Ki values for each standard sample.

$$K(T) = C \times (1 + dT + eT2) \quad \ldots 3.4$$

3.5.2.5 HSDSC Slope Correction

When HSDSC baseline was slanted, the slope can be found from the data and corrected by inputting the slope in HSDSC slope. The initial value for the HSDSC slope was 0 before making the measurement. The baseline slope correction was carried out without pan within the experimental temperature range (-20 - 100 ºC) and scanning rate of 10 ºC/min.

The DSC slope value was found using the following equation:

$$DSCslope = S' - \frac{y_2 - y_1}{T_2 - T_1} \quad ..3.5$$

where;

S’ = DSC slope value at time of measurement = 0 initial value

T_1, T_2 = temperature at 2 points on the baseline

y_1, y_2 = DSC reading at T_1 and T_2

3.6 Optimum Operating Condition Procedure Step-scan Mode

Instead of continuously heating the sample, heat is applied in stepscan-mode. Step-scanning mode was applied to all analysis, tested using sealed pan. Improved scanning rate was done in order to optimise the operating condition. Two criteria were used in the analysis; curve with the most significant endothermic peak according to the best fit of three repeated runs (especially the T_m value) and with acceptable statistic based on the lowest percentage of Absolute Average Deviation (AAD). The experiments were performed using 6-gingerol standard from Wako Pure Chemical Ind. Ltd. and pure glycerol fisher scientific sample, which is the characteristic as same as ginger oleoresin.

In order to confirm the accuracy of the instrument, enthalpy of fusion and melting temperature values of some organic compounds (glycerol and 6-gingerol standard) were determined and these values were compared with the literature. About 13.00 mg – 14.00 mg samples were weighed into aluminium pan and crimped. The heating rate was varied to identify the optimum scan rate from 10, 20, 50 and 100 ºC/min and the curve were recorded continuously from -20 to 100 ºC for both sample. Both samples were cooled and the temperature was held for 3 minutes to reach a steady state at -20 °C before it was scanned at the respective scanning rate up to 100 °C. At 100 °C, the temperature was again held for 3 minutes to reach a steady state. For each scanning rate, the heat flow measurements were repeated 3 times.

3.7 HSDSC Calculation

Melting point and heat of fusion can be determined from the 'HSDSC Analysis' job. Each analysis was obtained from the calculation as described below.

3.7.1 Melting Point Calculation, T_m

The 'transition point' job is used to read the time or temperature at the start of an endothermic HSDSC peak. The cursor was moved to the HSDSC T_m job icon in the 'HSDSC Analysis' menu. The transition-point reading job was selected. Two points (one on the data baseline and one on the peak slope) were specified with the line cursor. The temperature or time at the intersection between the tangential lines from the specified points gave the calculated transition point. A HSDSC melting peak is presented in **Figure 3.3.**

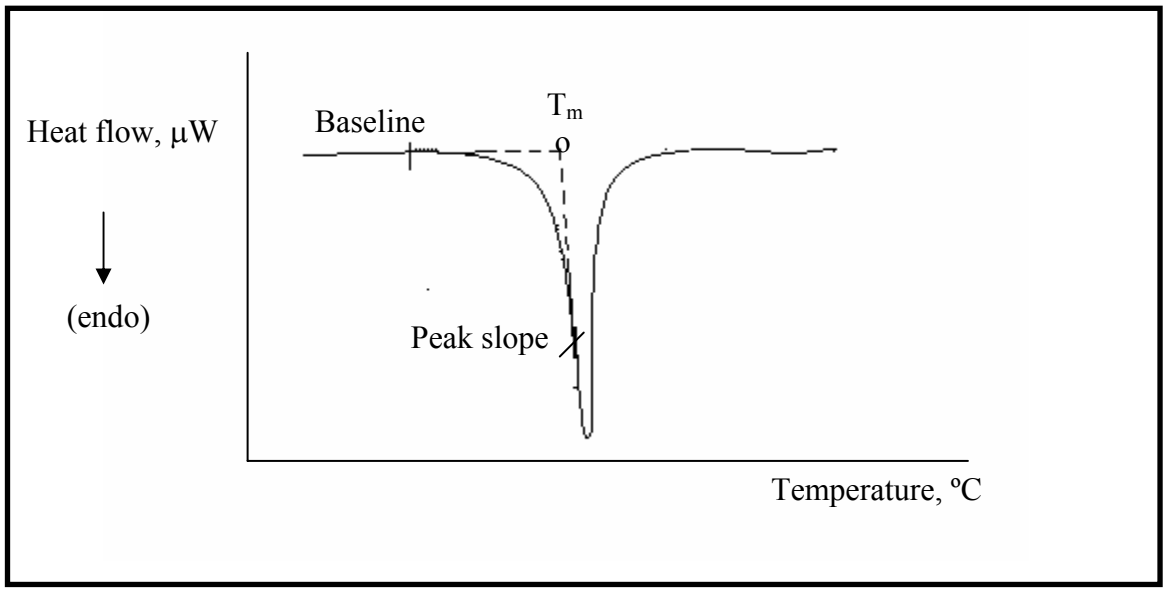


Figure 3.3: DSC peak showing the construction of the extrapolated onset

3.7.2 Heat of Fusion Calculation, ΔH_f

The ΔH_f job calculates the amount of heat from the area of the DSC peak, divides it by the sample weight to convert it into ΔH_f (mJ/mg) value. The procedure is given below.

The ΔH job was selected from the icon in the 'DSC Analysis' menu. Two points (one before and one after the peak) were specified with the line cursor. A straight line was drawn between those two points and the ΔH_f (mJ/mg) value was calculated and displayed for the area of the peak marked of by the straight line (**Figure 3.4**).

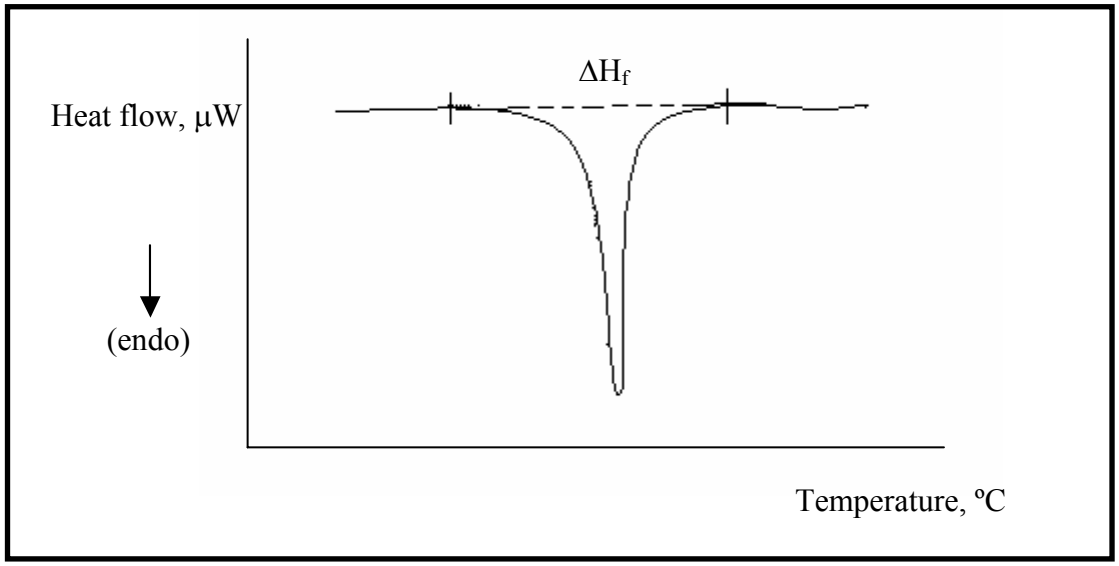


Figure 3.4: DSC measurement of heat of fusion

3.7.3 Purity Determination

The temperature of phase changes and reactions as well as heats of fusion were used to determine the purity of the compounds. **Equation 3.6** namely Van't Hoff equation is used:

$$\ln x_A = \ln(1 - x_B) = \frac{\Delta \overline{H}_{f,A}}{R}\left(\frac{1}{T_0} - \frac{1}{T}\right) \qquad \textbf{...3.6}$$

where;

x_A = mole fraction purity (%)

x_B = mole fraction impurity (%)

T_0 = theoretical melting point of the pure substance (K)

$T = T_m$, represent the melting point of impure sample (K)

$\Delta\overline{H}_{f,A}$ = heat of fusion of the major component, A (J/mol)

R = gas constant, 8.314 J/mol.K

3.8 HSDSC Data Analyze or Processing

This section will brief all procedures performed in this research. Data processing involved analysis of thermogram curves using *Pyris Perkin Elmer* software, give all the curves were in "*.pdid*" format. The data were analyzed to give the characteristics of the substances eg. the melting point and heat of fusion.

Altering or modifying method can be made on method editor dialog box and choose sample info. **Figure 3.5** shows sample info dialog box, which is used to set sample identification, name of the operator, set the directory and set the sample mass where the heat of fusion will be analyze. Then, to know whether the analysis action is going to start or occur immediately or not, click initial state dialog box at the method editor menu, **Figure 3.6**. After all info had been set, click on Program menu, the dialog box will appear as in **Figure 3.7**, which is used to select heating or cooling, continuous scans or step-scan and arrange method in order. Once a set of method is confirmed, click end condition and start at the DSC Control Panel Box. When analyzed sample start, click view program at the method editor menu as **Figure 3.8**, it is used to overview all parameters or variables that has been selected

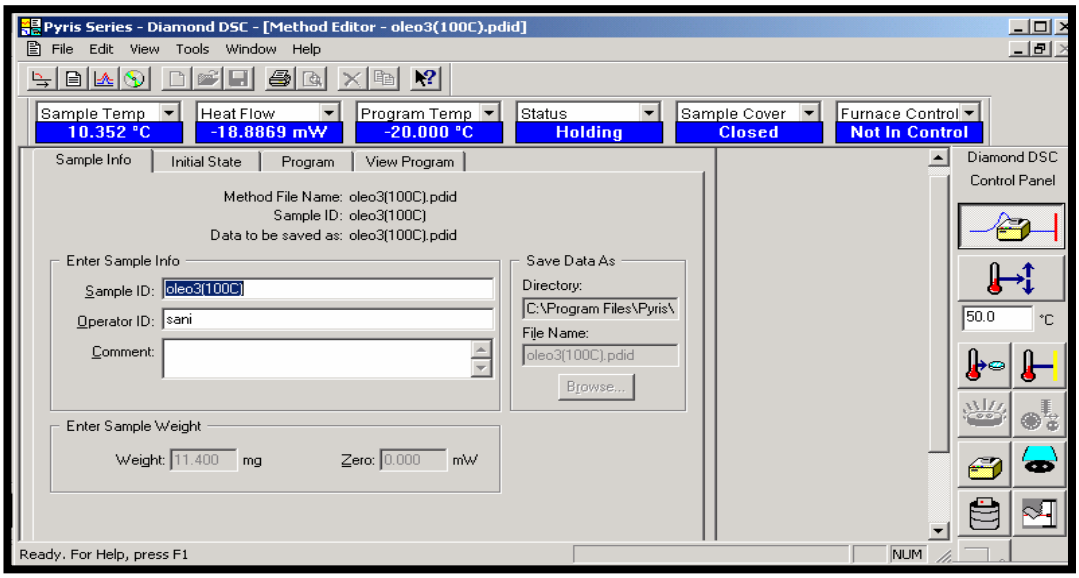


Figure 3.5: Sample info dialog box.

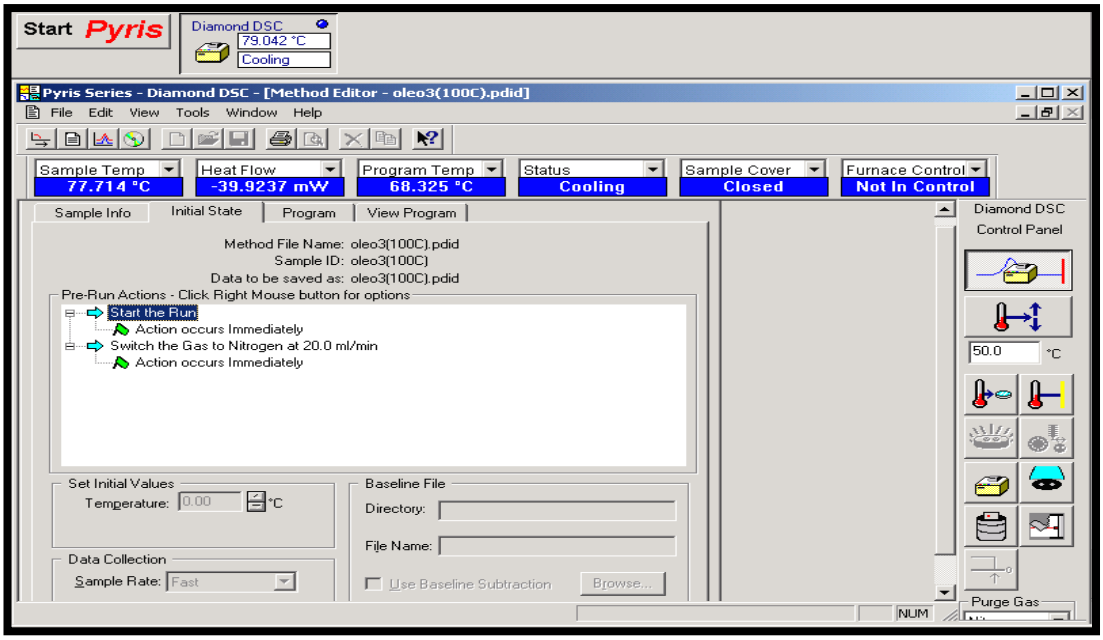


Figure 3.6: Initial state dialog box.

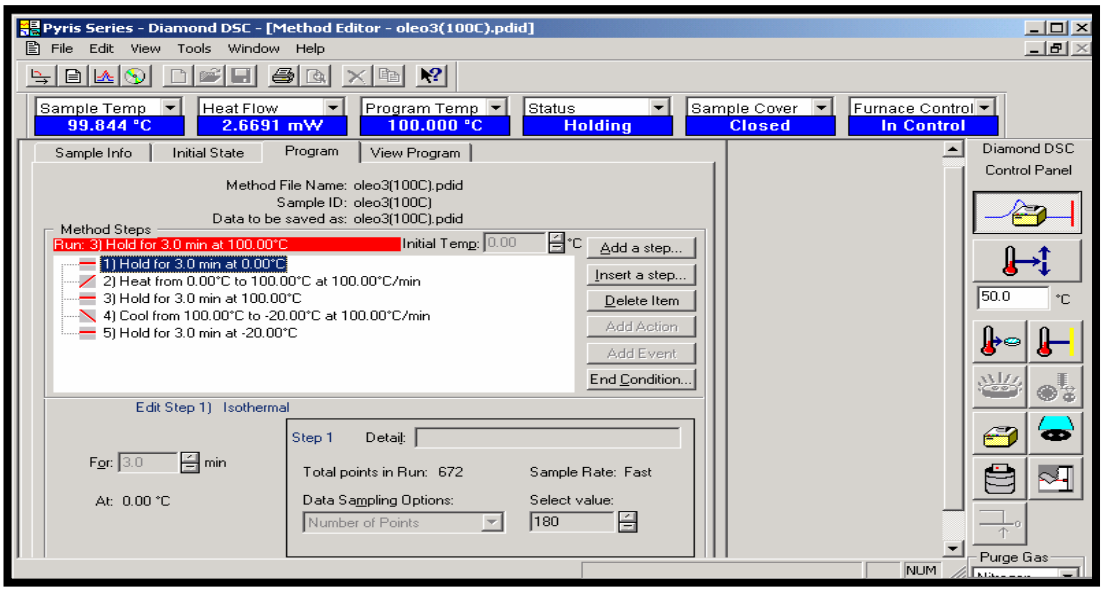


Figure 3.7: Program dialog box.

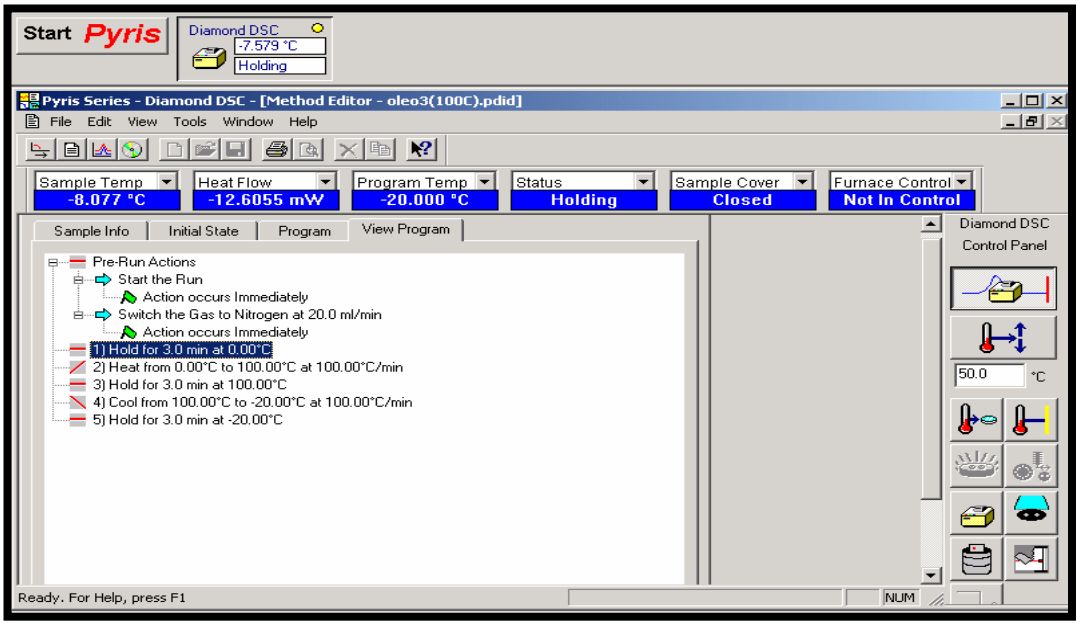


Figure 3.8: View program dialog box.

3.8.1 Melting Point and Heat of Fusion Analysis

Figure 3.9 shows a typical thermogram curve. First step, click the icon "Peak Search" on the menu bar, to detect and identify a peak along the curve.

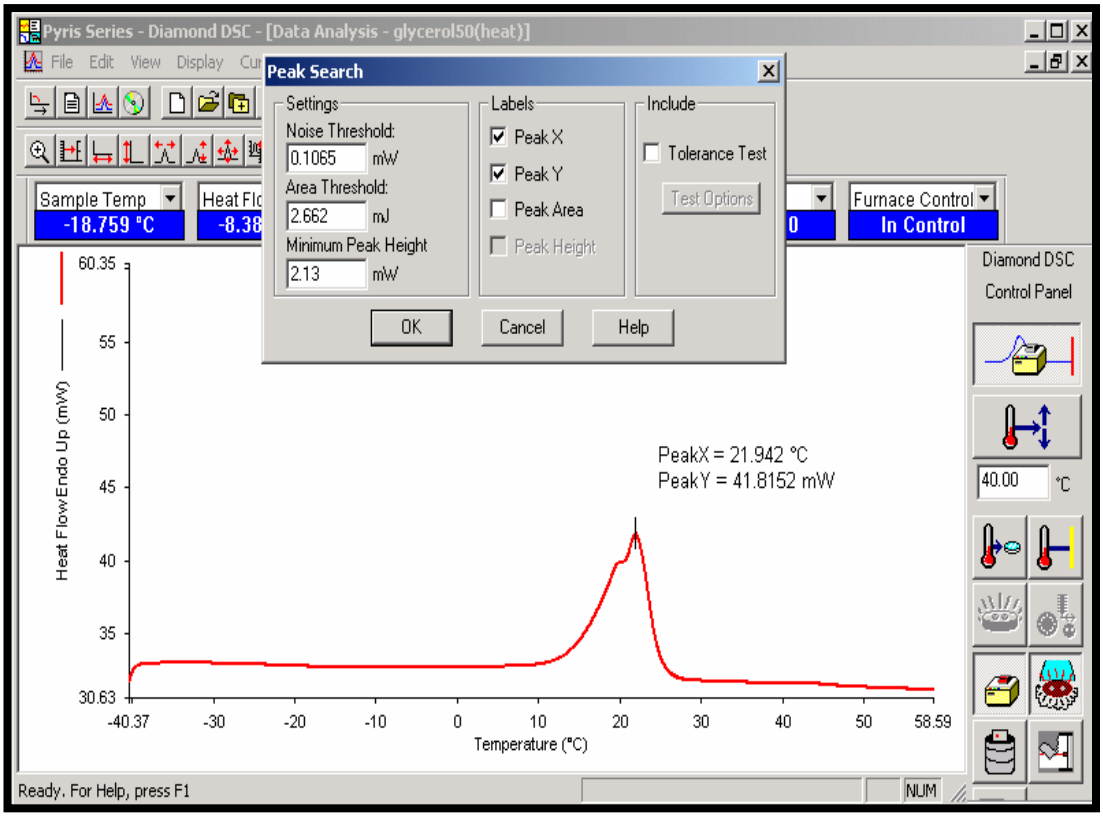


Figure 3.9: Peak search analysis dialog box.

Second step, click the icon "Peak Calculation" on the menu bar, **Figure 3.10**. Optionally, choose selection parameter onset, end and peak height at the peak calculation dialog box. Then click calculate, it will display all the parameter that had been selected.

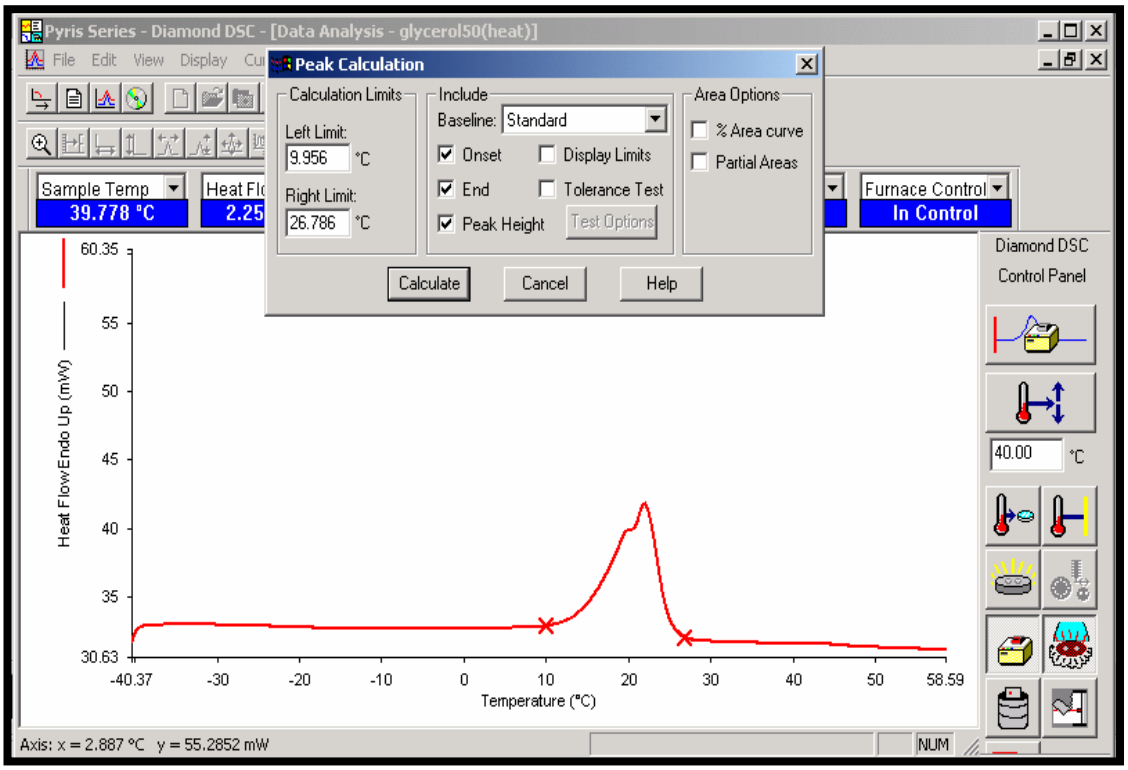


Figure 3.10: Peak calculation analysis dialog box.

The result including melting point and heat of fusion is displayed as in **Figure 3.11.**

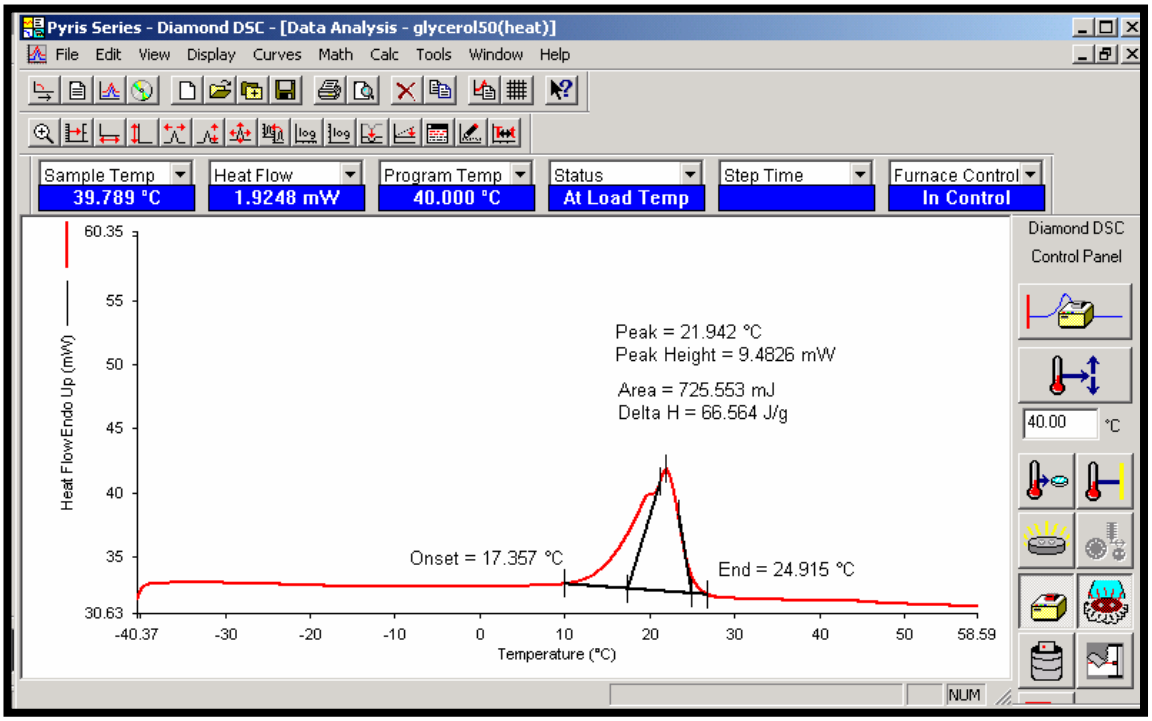


Figure 3.11: Result dialog box.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 *Zingiber officinale* Roscoe - Oleoresin

4.1.1 Yield of Ginger Oleoresin

The dried and grind rhizomes of *Z. Officinale* Roscoe (2500.00 g) were extracted with ethanol 98% (10 Liter) in a solvent extractor for 6 hours. The solvent was evaporated using Buchi R205 rotary evaporator to give the crude extract to yield ginger oleoresin, a brown viscous liquid (247.63 g) i.e. 9.91 % yield. Oleoresin is a mixture of a resin and an essential oil. The simple screening test was carried out to measure compound purity, either melting point for solid or refractive index measurement for liquid. The melting point of a solid sample was determined by HSDSC Perkin Elmer while the refractive index of liquid samples were measured using ATAGO's Abbe Refractometer 1T, accurate to ± 0.0002. The pure compounds were then investigated by spectroscopic and chromatographic methods for structural elucidation and confirmation of the compounds. A gas chromatogram of the ginger oleoresin is presented in **Appendix 1.**

4.1.2 6- Gingerol Isolation and Purification Techniques

Ginger oleoresin (14.00 mg) was subjected to gravity column chromatography over 10 g silica gel Merck 60 (70 - 230 mesh) in a slurry with the increasing polarity of mobile phase until PE:Et_2O (4:6 v/v). The slurry mixture was poured into the vertically oriented glass column (30 cm x 1.5 cm i.d.) to yield 41 fractions.

Fraction 37 was evaporated under vacuum to give compound 6-Gingerol (9.29 mg, 0.01%) as a pungent, dark brownish viscous liquid with R_f 0.28 by TLC in PE:Et_2O (1:1 v/v). IR ν_{max} cm^{-1}: 3435.0, 2927.7, 1708.8, 1515.9, 1269.1; ^1H NMR ($CDCl_3$): δ 0.85 (3H, t, *J* = 7.0 Hz, Me), 1.20-1.55 (8H, m, H-9, H-8, H-7, H-6), 2.45 (1H, dd, *J* =17.2 Hz, 8.0 Hz, H-4), 2.70-2.80 (4H, m, H-1, H-2), 3.02 (1H, broad, s, OH), 3.85 (3H, s, OMe), 4.00 (1H, m, H-5), 5.55 (1H, broad, s, Ar-OH), 6.60 (1H, dd, *J* =8.0 Hz, 2.0 Hz, H-6'), 6.61 (1H, d, *J* =2.0 Hz, H-2'), 6.80 (1H, d, *J* =8.0 Hz, H-5'); ^{13}C NMR ($CDCl_3$): δ 14.00, 23.00, 25.50, 29.50, 32.00, 37.50, 46.00, 49.50, 56.00, 68.00, 111.00, 114.00, 121.00, 133.00, 144.00, 147.00, 212.00.

The IR spectrum (**Appendix 2**) showed a broad hydroxyl absorption at 3435.0 cm^{-1}, a strong carbonyl absorption at 1708.8 cm^{-1}, aromatic stretching at 1604.7 and 1515.9 cm^{-1} and also C-O stretching at 1269.1 cm^{-1}. **Table 4.1** gives the IR parameter of compound 6-Gingerol.

Table 4.1: IR Parameter for Compound 6-Gingerol.

Frequency (cm^{-1})	Intensity	Type of Bond
3435.0	M	O-H
2927.7 and 2858.3	S	C-H
1708.8	S	C=O
1604.7 and 1515.9	M	C=C aromatic
1269.1	S	C-O stretch

m = medium, s = strong

^{1}H NMR spectrum (**Appendix 3**) of this compound indicated that compound 6-Gingerol had a similar structure to compound Shogaol due to the presence of a methoxyl group at 3.85 (3H, s), methyl group at δ 0.85 (3H, t, *J*=6.3 Hz) and three aromatic protons (H-6', H-5' and H-2') observed at δ 6.60, 6.61 and 6.80 respectively. However, the spectrum for compound 6-gingerol showed a broad peak at δ 3.02 was attributed to the hydroxyl proton. A broad single peak integrating for two protons was assigned to two hydroxyl protons at δ 5.55. A multiplet peak at δ 2.70-2.80 was assigned for four protons (H-1 and H-2). Four methylene groups (H-9, H-8, H-7 and H-6) were observed at δ 1.20–1.55 as multiplet.

The ^{13}C NMR spectrum (**Appendix 4**) supported that compound 6-Gingerol based on the 17 peaks, which represents 17 carbons in the molecule. The presence of carbon methyl was observed at δ 14.00 and δ 56.00, seven methylene carbon, C-1 (29.5), C-2 (46.0), C-4 (49.5), C-6 (37.0), C-7 (32.0), C-8 (25.5), C-9 (23.0) and metyna C-5 (68.0), carbonyl carbon C-3 (212.0), quaternary carbon C-1' (133.0), C-3' (147.0), C-4' (144.0) and carbon aryl C-2' (111.0), C-5' (114.0) and C-6' (121.0). That compound has been identified as compound 6-Gingerol based on the physical properties and the spectroscopic data of compound isolated from *Zingiber officinale* Roscoe [4] and compared with the authentic sample and compound 6-Gingerol standard. Both of the NMR data are summarised in **Table 4.2.**

Table 4.2: ^{1}H NMR and ^{13}C NMR Parameters for Compound 6-Gingerol.

Carbon	^{13}C, δ (ppm)	^{1}H, δ (ppm)
1	29.50	2.70-2.80
2	46.00	2.70-2.80
3	212.00	
4	49.50	2.45, 2.50
5	68.00	4.00
		3.02 (-OH)
6	37.00	1.20-1.55
7	32.00	1.20-1.55
8	25.50	1.20-1.55
9	23.00	1.20-1.55
10	14.00	0.85
1’	133.00	
2’	111.00	6.61
3’	147.00	
4’	144.00	5.55
5’	114.00	6.80
6’	121.00	6.60
Ome	56.00	3.85

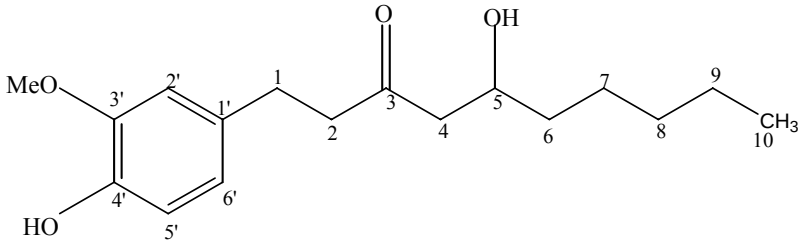


Figure 4.1:- Gingerol structure.

(1-(4’-Hydroxy-3’-methoxyphenyl)-5-hydroxy-3-decanone)

4.2 Purity Determination for Organic Compound Analysis Using DSC

6-gingerol is a relatively stable compound that does not readily decompose unless under extreme conditions. Hence, the DSC temperature studied was at low temperature, less than 100°C. Figure 4.2, shows the DSC peak of isolated and standard 6-gingerol. Both samples melt below 30°C and the broad peak was observed due the nature of the sample, which is amorphous solid. The T_m and ΔH_f data of 6-gingerol samples are presented in Table 4.3.

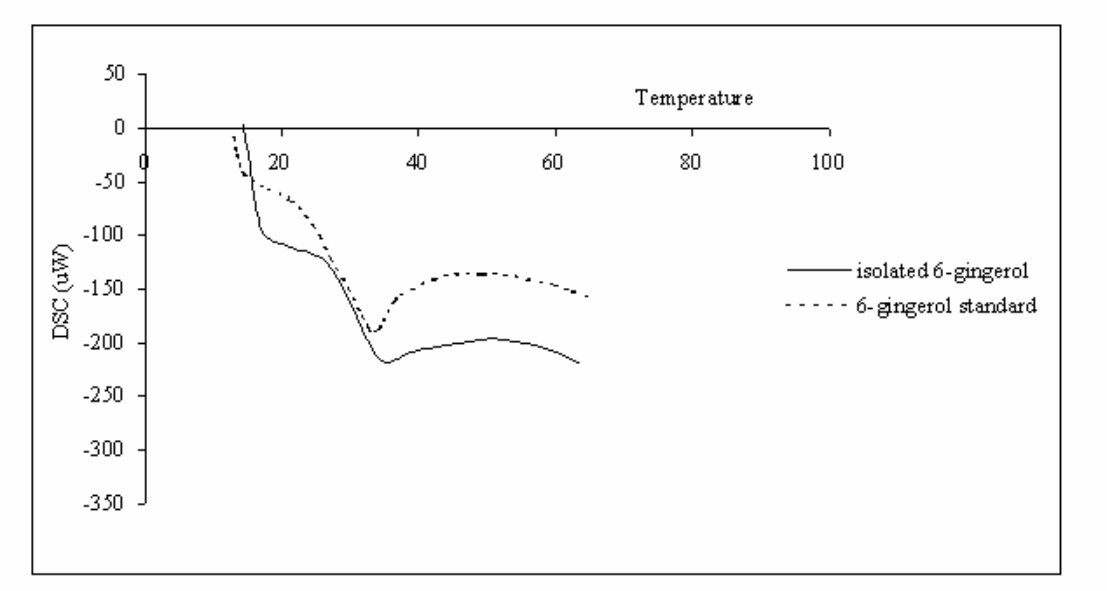


Figure 4.2: Comparison of DSC scan for isolated and standard of 6-gingerol.

Table 4.3: T_m and ΔH_f data of 6-gingerol

Sample	Sample mass (mg)	Melting temperature, T_m (°C)		Heat of fusion, ΔH_f (mJ/mg)	DSC % mole purity
		Onset	Peak maximum		
Compound (**1**) std. (Wako product)	1.00	26.20	34.50	50.6	93.24
Isolated compound (**1**)	1.02	29.20	36.88	18.7	99.34

From the graph (Figure 4.2), the fusion occurred endothermically in the temperature range from 25 to 50 ºC. The samples identified as 6-gingerol based on their melting points and not on the heat of fusion data. Isolated 6-gingerol reported in this study show a temperature onset very close to the literature melting point.

For 6-gingerol standard, the melting point was 26.20 ºC and its heat of fusion was found to be 50.6 mJ/mg. For the isolated 6-gingerol the melting point was indicated at 29.20 ºC and its heat of fusion was found lower than the standard, which was 18.7 mJ/mg. T_m data was not much different, except for heat of fusion where the difference was quite obvious. However, from DSC purity calculation, both compounds showed more than 90 % purity. It showed that purity determination by DSC is depends on T_m value rather than ΔH_f value.

4.3 HSDSC Scans for Organic Compounds

Organic compounds, glycerol which melts at the same temperature range with the investigated samples 6-gingerol, were tested using the calibrated DSC. The accuracy of the instrument was checked by determination of melting temperature and heat of fusion for each pure organic compound. Pure glycerol (Fisher Scientific) is a viscous liquid, similar condition to the investigated sample, ginger oleoresin. Pure 6-gingerol standard (Wako Pure Chemical Ind. Ltd.) is a major constituent of ginger oleoresin responsible for ginger's characteristic.

4.3.1 Optimum Conditions for Pure Organic Compounds Analysis

An alternative step-scan mode was used in this approach; the HSDSC was programmed to make a series of small temperature interval, 1ºC each followed by an isothermal period. Subsequent temperature steps and holding periods follow, until the whole temperature range of interest was examined. This scanning mode is called 'step-scan mode'. Step-scan technique can be applied to all substances that are thermally stable during melting in order to analyze more peaks instead of one broad peak [23]. This may be particularly advantageous if the melting is close in

temperature to one. It is allowing sufficient time for a melting to occur within a small temperature interval and also between steps for heat flow to reach a steady state [66].

Hence, much greater time was required to analyse sample using step-scan compared to continuous scan. Fortunately, if the analysis uses higher scan rate it will definitely take much less time. The experiments were conducted at step-scan mode and various scanning rates (10, 20, 50 and 100 °C/min). The sample mass was kept constant between 13.0 mg to 14.0 mg for all applied scan rates, tested using sealed aluminium pan. Due to that, statistical analysis was explored using Microsoft office-Excel spreadsheet to get the percentage Average Absolute Deviation (AAD). The lowest percentage was favoured, since this indicates a small gap between literature and experimental data, indicating good agreement between experimental and literature results.

4.3.2 6-Gingerol Step-scan Analysis

6-Gingerol is a relatively stable compound that does not readily decompose unless under extreme conditions [65]. Hence, the HSDSC temperature studied was at low temperature, less than 100 °C. From **Figure 4.3**, each scan rates 10 and 20 °C /min for 6-Gingerol standard showed only one broad endothermic peak and the melting peak was not clearly defined. The appearance of broad peak is due to the nature of the sample itself, where an amorphous compound or viscous liquid is melted, there is no sharp change in structural characteristic, but rather a gradual lost of interactions [64]. At the fastest scanning rate of 100 °C /min, the peak is very sharp but the melting point value was little bit far from literature. **Table 4.3**, the optimum scan rate that gives nearest melting peak value is 50 °C /min at 28.93 °C.

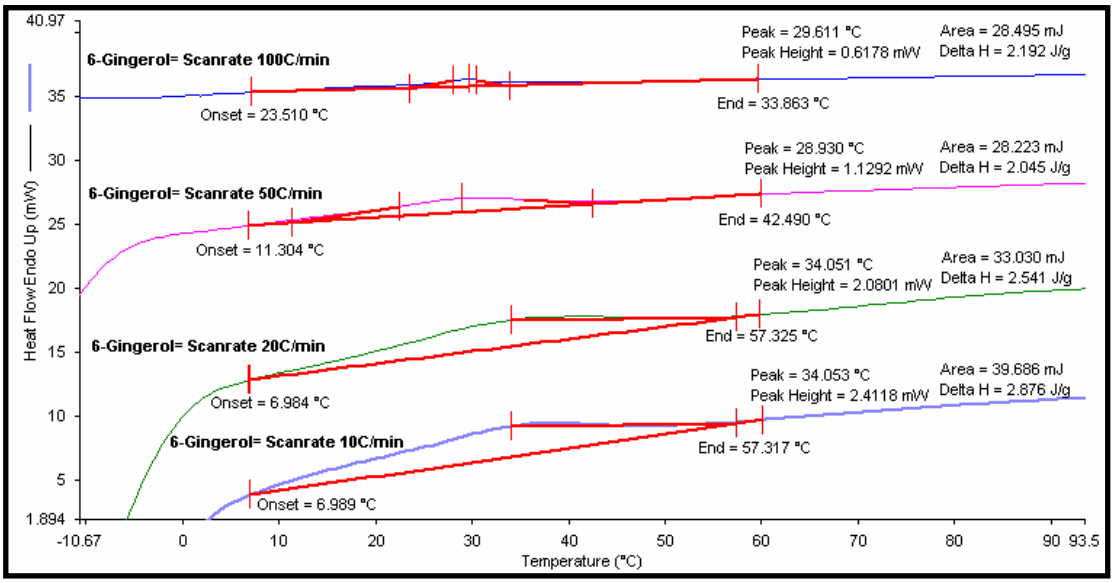


Figure 4.3: DSC Heat Flow of 6-Gingerol Standard as a Function of Temperature at Varied Scan Rates 10, 20, 50 and 100 ºC/min, Sample Mass: 13.8 mg.

Table 4.4: Melting point variation of 6-Gingerol Standard As A Function of Varied Scan Rates.

Scan Rate (°C /min)	Melting Point, Tm (°C)	AAD [(Tm-Tr)/Tr]	AAD %
100.00	29.61	0.013	1.3
50.00	28.93	0.036	3.6
20.00	34.05	0.135	13.50
10.00	34.05	0.135	13.50

Tr = Reference point = 29.00 °C

Tm = Melting point

AAD = Average absolute deviation

4.2.3 Glycerol Step-scan Analysis

Pure Glycerol thermal behaviour was monitored using HSDSC. The range of temperature -20°C to 100 °C was chosen since its melting point is just below 30°C.

Figure 4.4, illustrates that the melting point 18.14°C at scan rate 50 °C /min was estimated the nearest to the literature value at 0.30% AAD in **Table 4.5**.

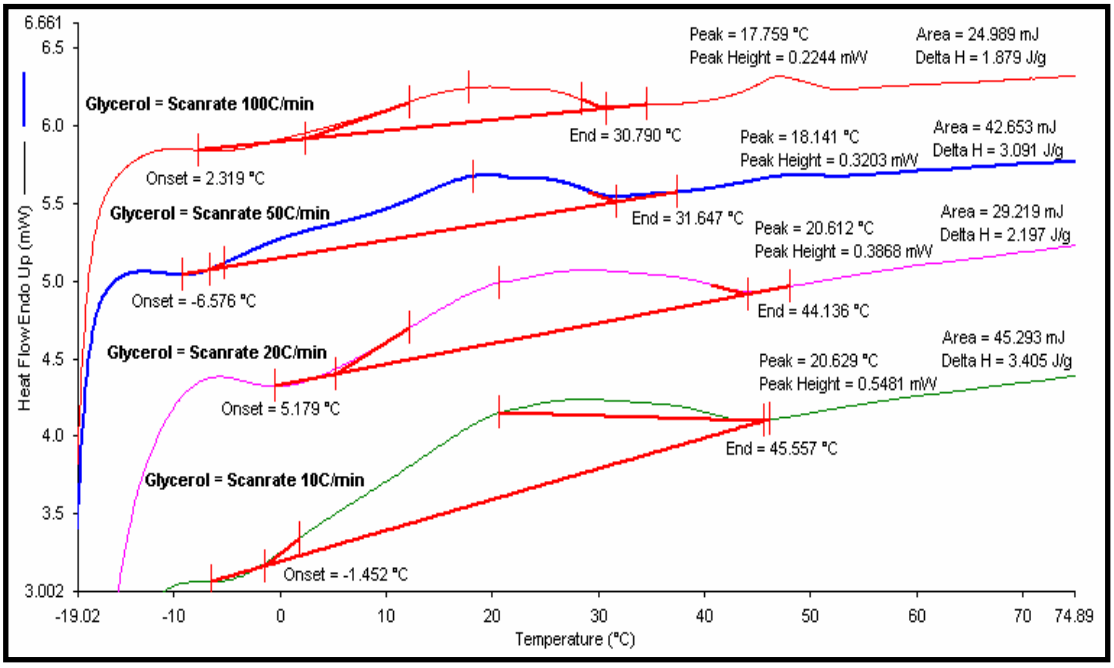


Figure 4.4: DSC Heat Flow of Glycerol as a Function of Temperature at Varied Scan Rates 10, 20, 50 and 100 ºC/min, Sample Mass: 13.8 mg

Table 4.5: Pure glycerol as a function of scan rates – Tm variation with varied scan rate.

Scan Rate (°C /min)	**Melting Point, Tm (°C)**	**AAD [(Tm-Tr)/Tr]**	**AAD %**
100.00	17.76	0.024	2.40
50.00	**18.14**	**0.003**	**0.30**
20.00	20.61	0.133	13.30
10.00	20.63	0.133	13.30

Tr = Reference point = 18.20°C [67]

Tm = Melting point

4.3.4 Optimum Condition for Analyzing Ginger Oleoresin

The results from **Figures 4.3, 4.4 and 4.5** were summarised in **Table 4.6.** This gives the experimental conditions as well as the measured results for organic compounds. The melting points of these organic compounds determined by HSDSC were in good agreement with the literature values except for the heat of fusion, ΔH_f (J/g).

Ginger oleoresin is a semisolid mixture or amorphous solid compound of a resin and essential oil, constituent of compounds 6-Gingerol, 6-Shogaol, Zingerone and Zingeberene. The oleoresin on scanning gives the broad melting peak. The optimum conditions were identified after analysing pure glycerol and 6-Gingerol standard. Scan rate of 50 °C /min was chosen as the optimum value. The range of temperature between -20 to 100 °C and sample mass ranging from 13.0 mg to 14.0 mg were used for ginger oleoresin.

Table 4.6: Experimental Conditions and Results Applied to Organic Compounds After Calibration.

Organic compounds	Sample mass (mg)	Scan rate (°C/min)	Temp. Range (°C)	Melting point, T_m (°C)		Heat of fusion, ΔH_f (J/g)	
				Exp.	Lit.	Exp.	Lit.
Glycerol	13.8	50.0	-20.0 -100.0	18.14	18.20[67]	3.091	200.62[67]
6-Gingerol Standard (Wako Pure Chem. Ind. Ltd)	13.8	50.0	-20.0-100.0	28.93	29.00[43]	2.045	50.6[44]
Ginger Oleoresin	13.8	50.0	-20.0 -100.0	27.90	30.34[44]	2.055	72.4 [44]

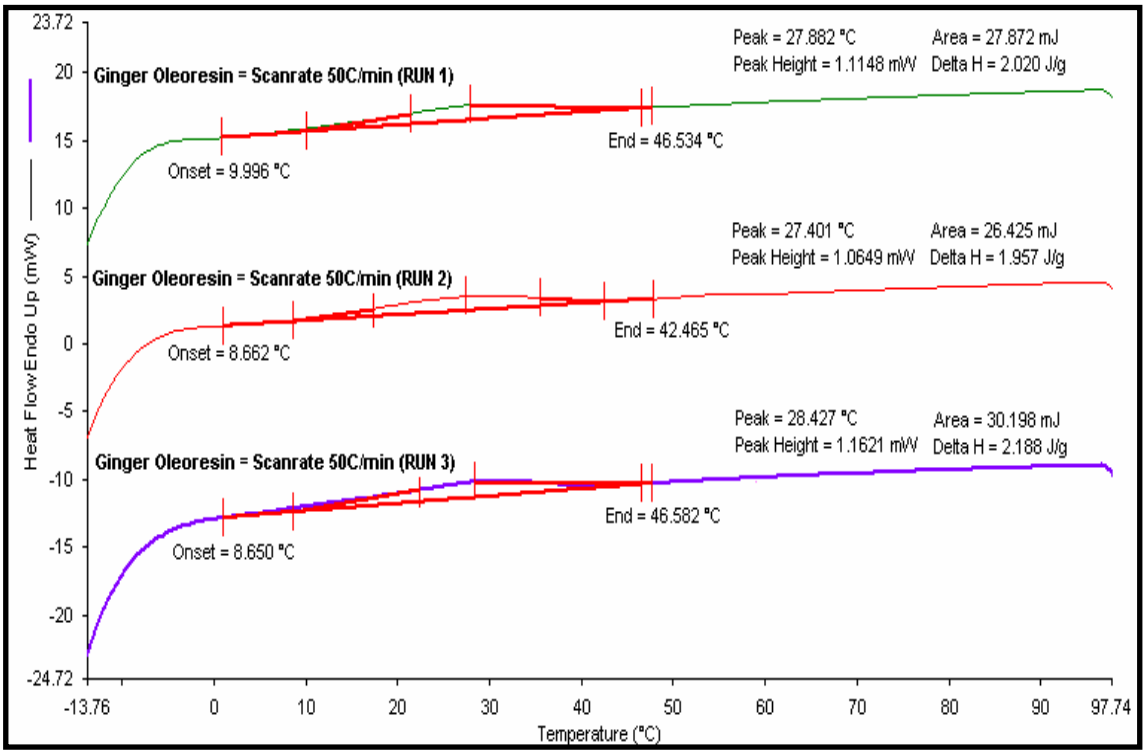


Figure 4.5: DSC Heat Flow of Ginger Oleoresin as a Function of Temperature At Scan Rate 50ºC/min,
Sample Mass: 13.8 mg.

Table 4.7: Ginger Oleoresin at Scan Rate 50 ºC/min.

Scan Rate (°C /min)	Melting Point, Tm (°C)	Coefficient of Variation (%)
50.00 (1st Run)	27.88	-0.07
50.00 (2nd Run)	27.40	-1.79
50.00 (3rd Run)	28.43	1.90

$$\text{Coefficient of variation} = \left(\frac{T - T_{ave}}{T_{ave}} \right)$$

$T_{ave} = 27.90°C$

For this set of experiment, the goal was to get the precise ginger oleoresin melting point, its predicted value from previous researcher using heat flux Seiko DSC is 30.34 °C [44]. Analysis of ginger oleoresin based on the melting point can provide information about the major compounds. The melting points of gingerol,

shogaol and zingerone are 30 - 31 °C [44], less than 30 °C and 40 - 41 °C [44] respectively. From Figure 4.5, the broad peak is believed to cover a wide range of temperature so as to include the entire major component's melting point, which is close to each other. The melting of the mixed samples can be estimated from the HSDSC peaks. The average melting point of ginger oleoresin 27.90 ºC at 50 ºC/min is in good agreement with the melting point of 6-Gingerol standard as stated in the literature.

4.4 Heat of Fusion

The heats of fusion is the amount of energy required to the melt a gram of sample mass. The heats of fusion of 6 – gingerol and ginger oleoresin are as tabulated in Table 4.8.

Table 4.8 Heats of Fusion of 6 – gingerol and Ginger Oleoresin At The Optimum Scan Rate of 50°C/min (sample mass 13.0 – 14.0 mg).

Sample	DSC	ΔH_f (J/g)	ΔH_f (ave.)	Coeff. Of Variation(%)
6 – gingerol	Perkin Elmer Diamond (MARDI)	2.045		
ginger oleoresin	Perkin Elmer Diamond (MARDI)	2.020	2.055	- 1.703
		1.957		- 4.769
		2.188		6.472
ginger oleoresin	Perkin Elmer Diamond (ORIEM)	0.729		
	Mettler Toledo			
	RRI Sg.Buloh	0.37		

The heats of fusion of ginger oleoresin was found to vary significally, from one DSC to the others, therefore the results of ΔH_f are not conclusive. However, based on the HSDSC in MARDI, the ΔH_f of 6 – gingerol is 2.045 J/g whilst that of ginger oleoresin is 2.055 J/g.

4.5 Verification of Ginger Oleoresin Composition.

The heats of fusion of ginger oleoresin of 2.055 J/g is very close to those of 6 – gingerol at 2.0455 J/g. Based on Kay's rule for mixture, on a property $\sum x_i P$ where, x_i = mole fraction of a compound and P = property, the equation can be extended to heats of fusion.

Hence,

$$\sum x_i \, \Delta H_{fi} = \Delta H_{f(mixture)}$$

Taking mole fraction of 6 – gingerol to be x_a, the other components are $1 - x_a = x_b$.

$$\therefore \; x_a \Delta H_{fa} + (1 - x_a)\Delta H_{fb} = \Delta H_{f(mixture)}$$

This indicates that ginger oleoresin extracted in this work comprises mainly of 6 – gingerol. The other possibility is that ginger oleoresin may comprise of very similar substance to 6 – gingerol, which is not detectable by using HSDSC. Hence, the melting curve of ginger oleoresin is slightly broader than those of 6 – gingerol.

CHAPTER V

CONCLUSIONS

5.1 Conclusions

Based on the result, several conclusions can be drawn from the experiments conducted using DSC technique. Sample mass used in this research was about 13.0 mg. Higher mass was selected since it gave a smaller and closer to the true melting point. The sample was heated at a rate of 50 °C/min as used for instrument calibration and it was also valid for reproducible results in crude ginger oleoresin and pure 6-Gingerol analysis. The Power Compensated DSC and the High Sensitivity DSC was not really sensitive in detecting a few compounds in ginger oleoresin because of the broad peak and heat of fusion value differ from literature. Separation of major compounds in mixture can only be done in chromatographic techniques, but not in thermal analysis techniques. In complex mixture, ginger oleoresin showed only one broad endothermic peak. The melting point was corresponded to the 6-gingerol's compound. The broad peak was attributed to the multi-component mixed samples. It was assumed to cover a wide range of melting point of the major compounds, which are close to each other. Step-scan analysis was applicable for this kind of DSC, since continuous scan cannot detect or identify any thermal changes. The DSC however, can be used to identify purity of samples provided the standard sample data on melting point is available and the purity is above 80%.

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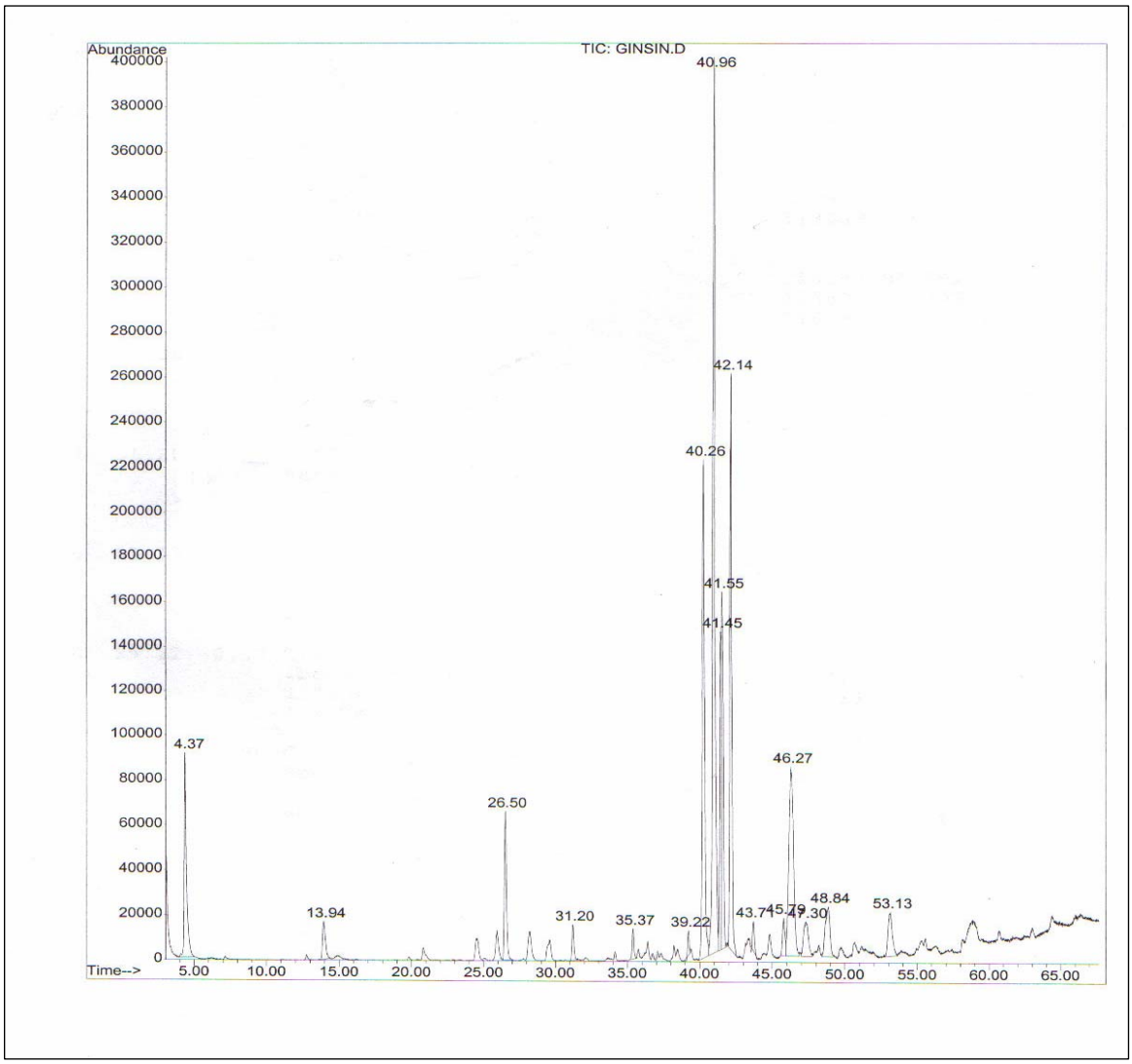
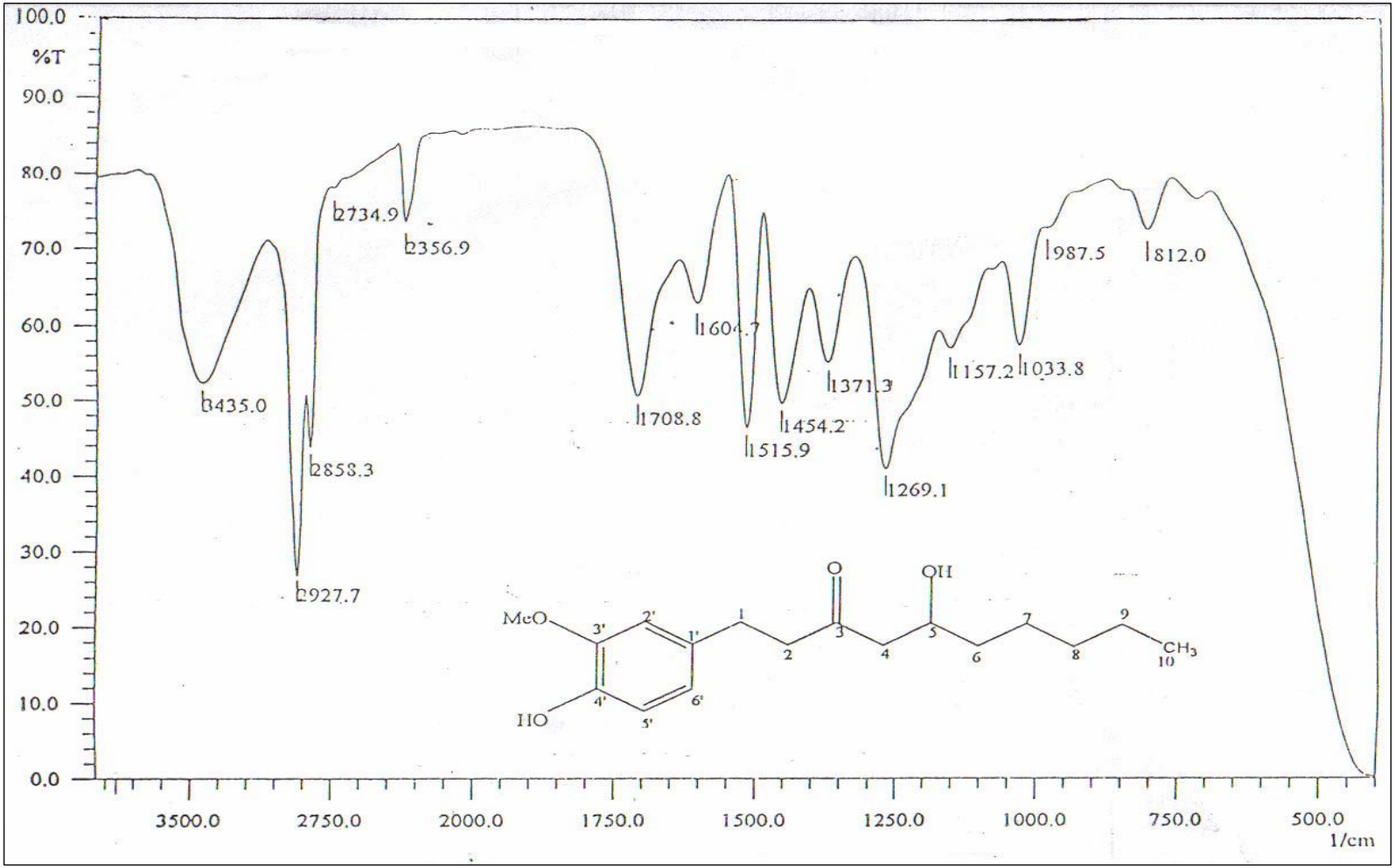
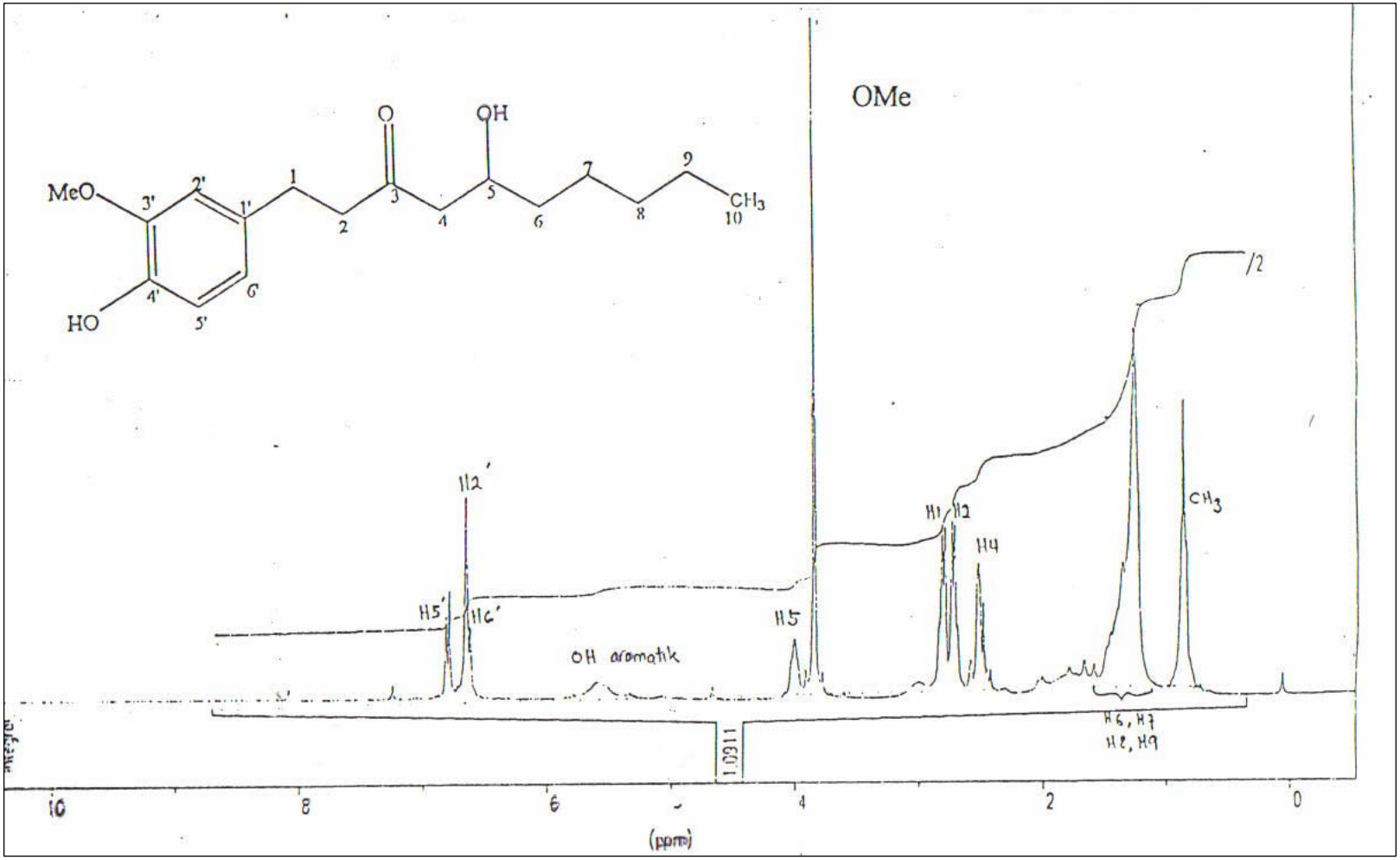
APPENDIXES



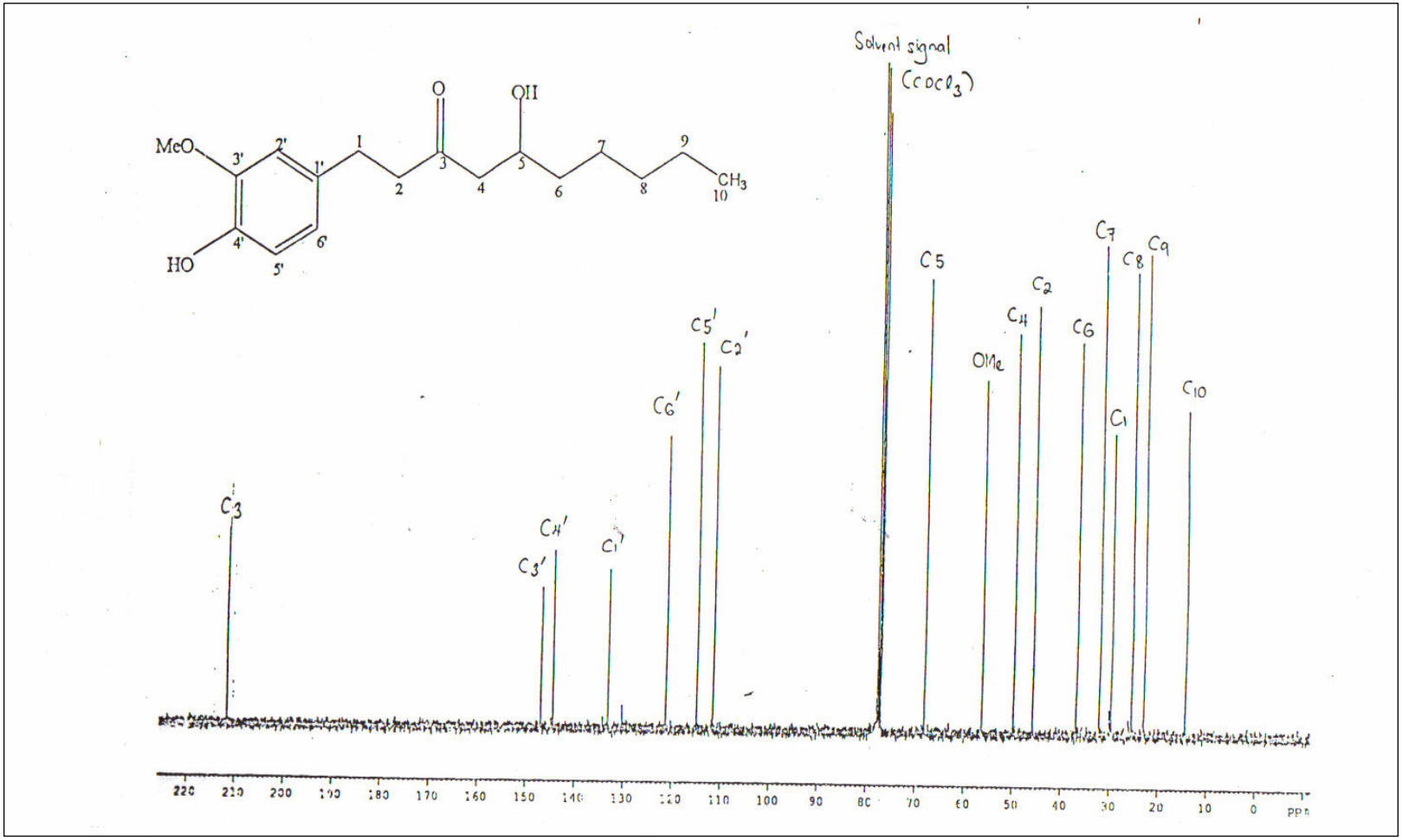
APPENDIX 1: Gas chromatograph of ginger oleoresin



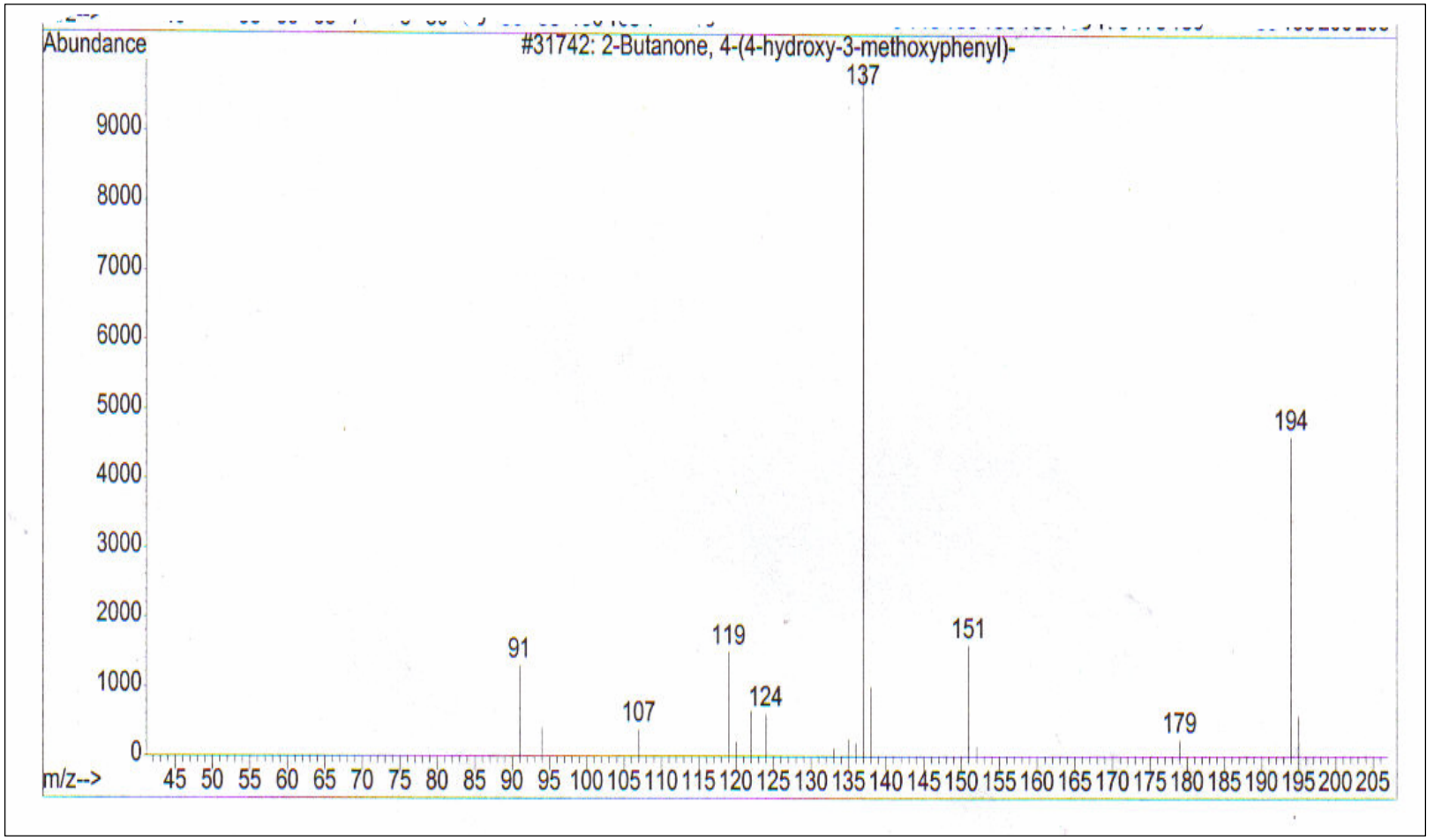
APPENDIX 2: IR spectrum of compound 6-gingerol



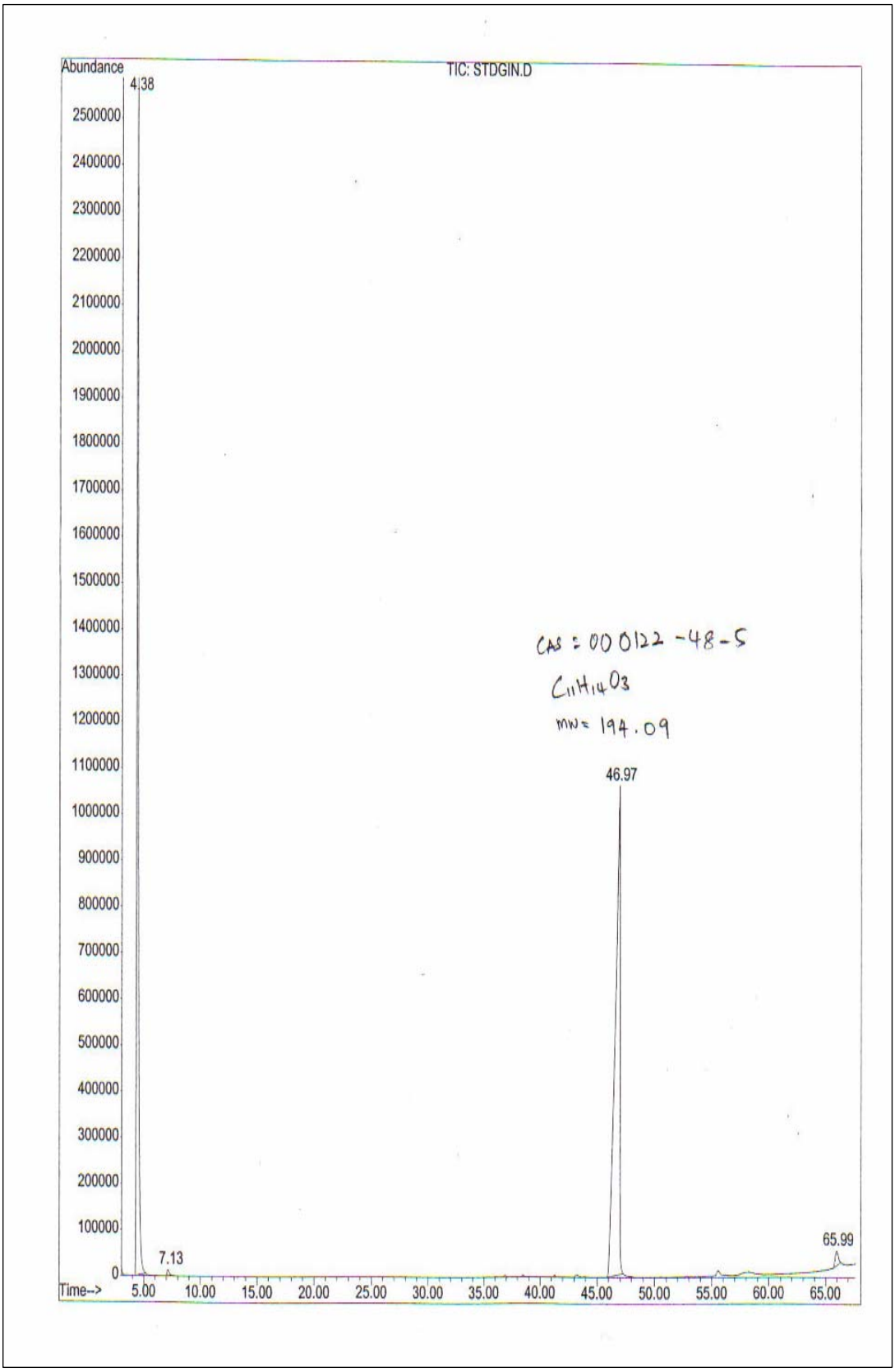
APPENDIX 3: ^{1}H NMR of compound 6-gingerol



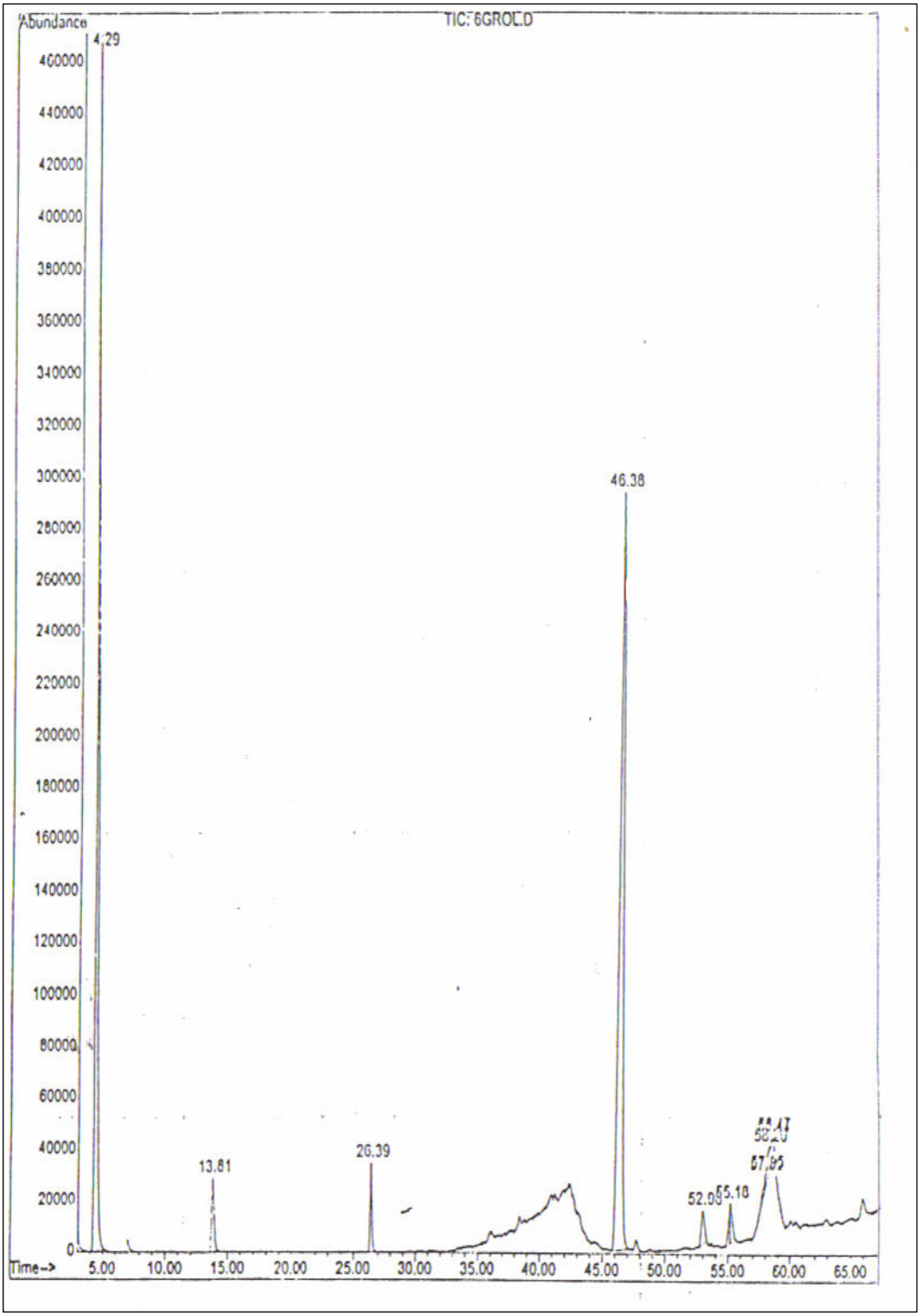
APPENDIX 4: ^{13}C NMR of compound 6-gingerol



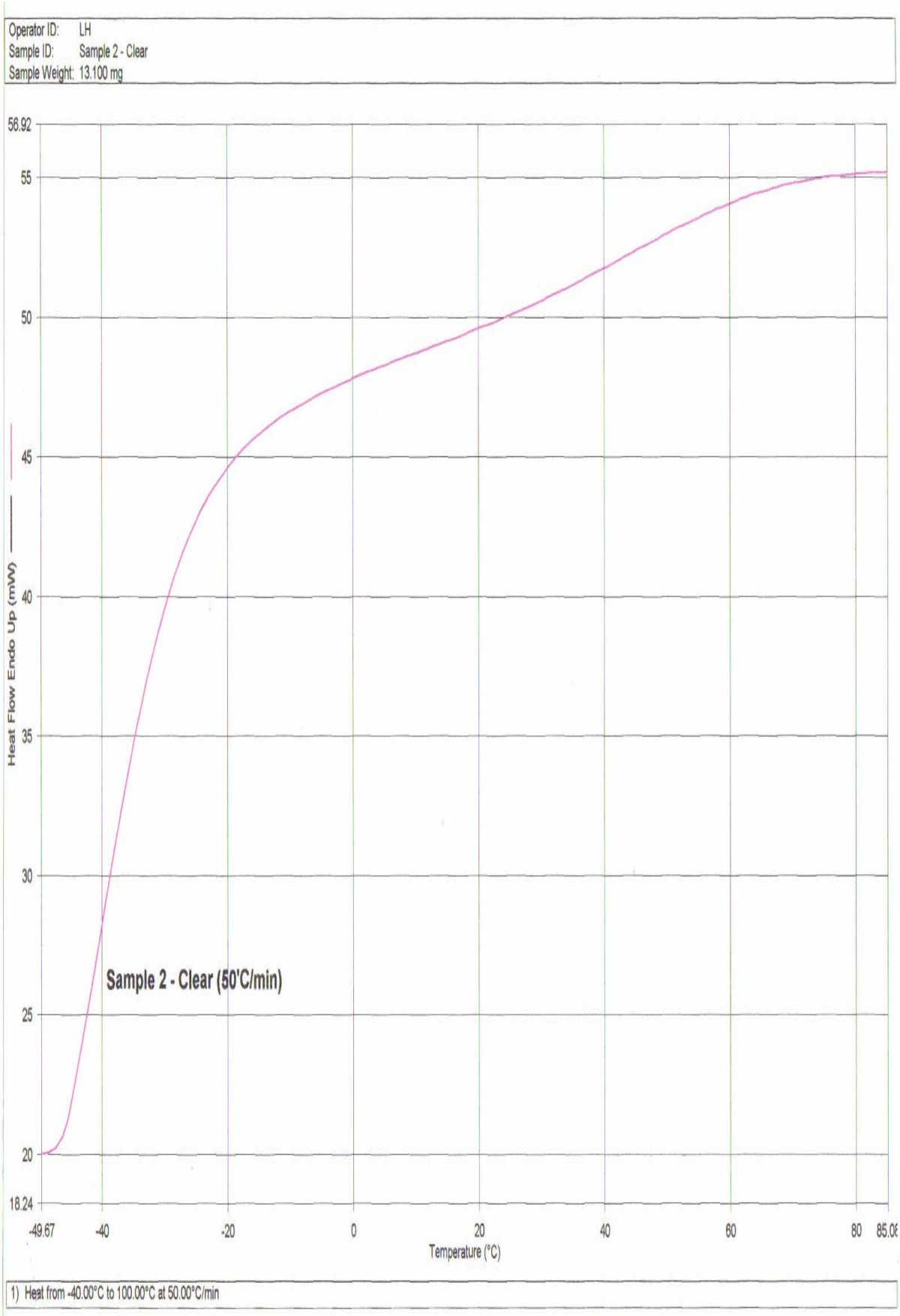
APPENDIX 5: MS of compound 6-gingerol (m/e = 194 $[M]^+$ (from 98% mole purity)



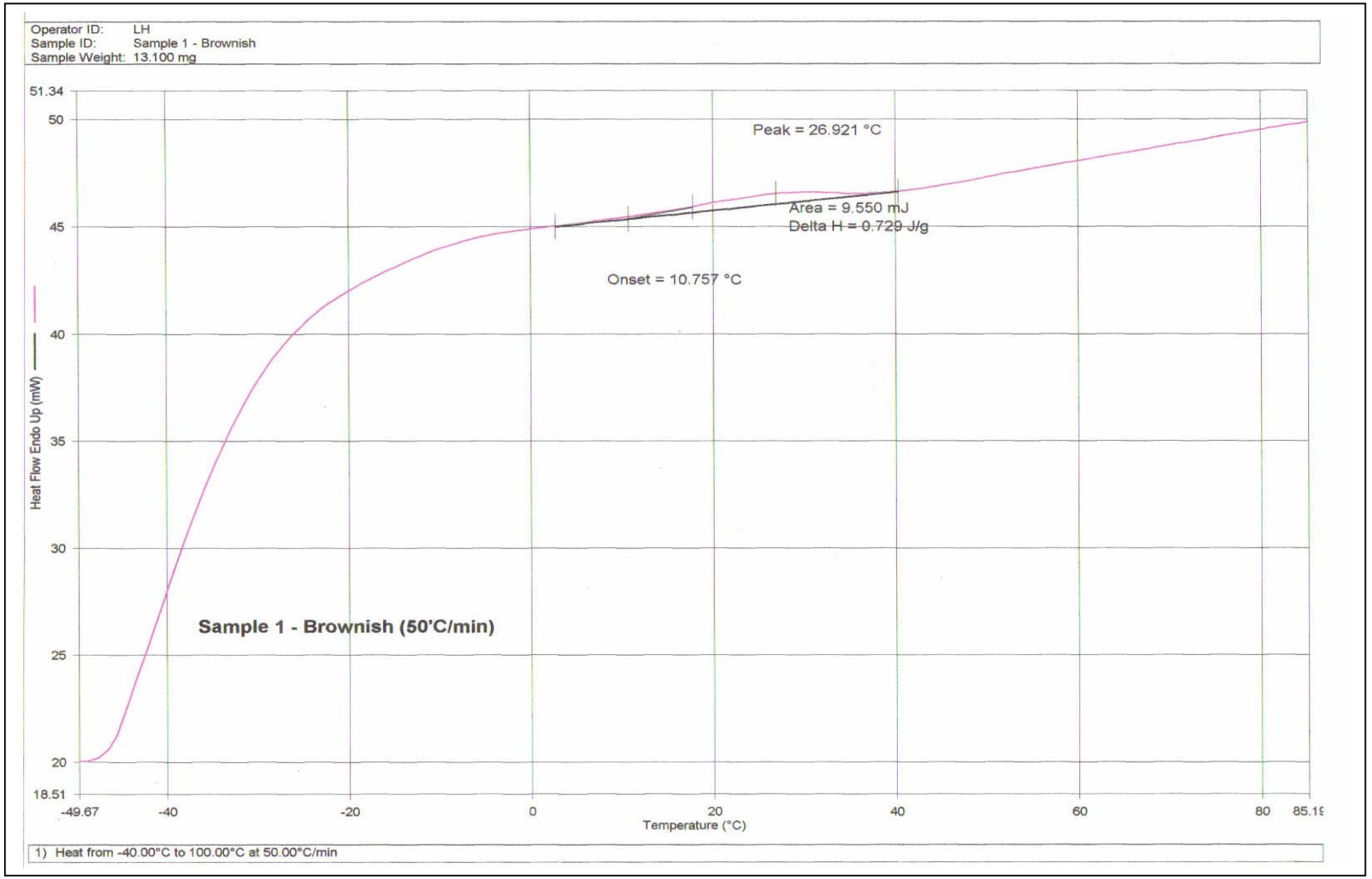
APPENDIX 6: GC-MS of compound 6-gingerol (from 98% mole purity)



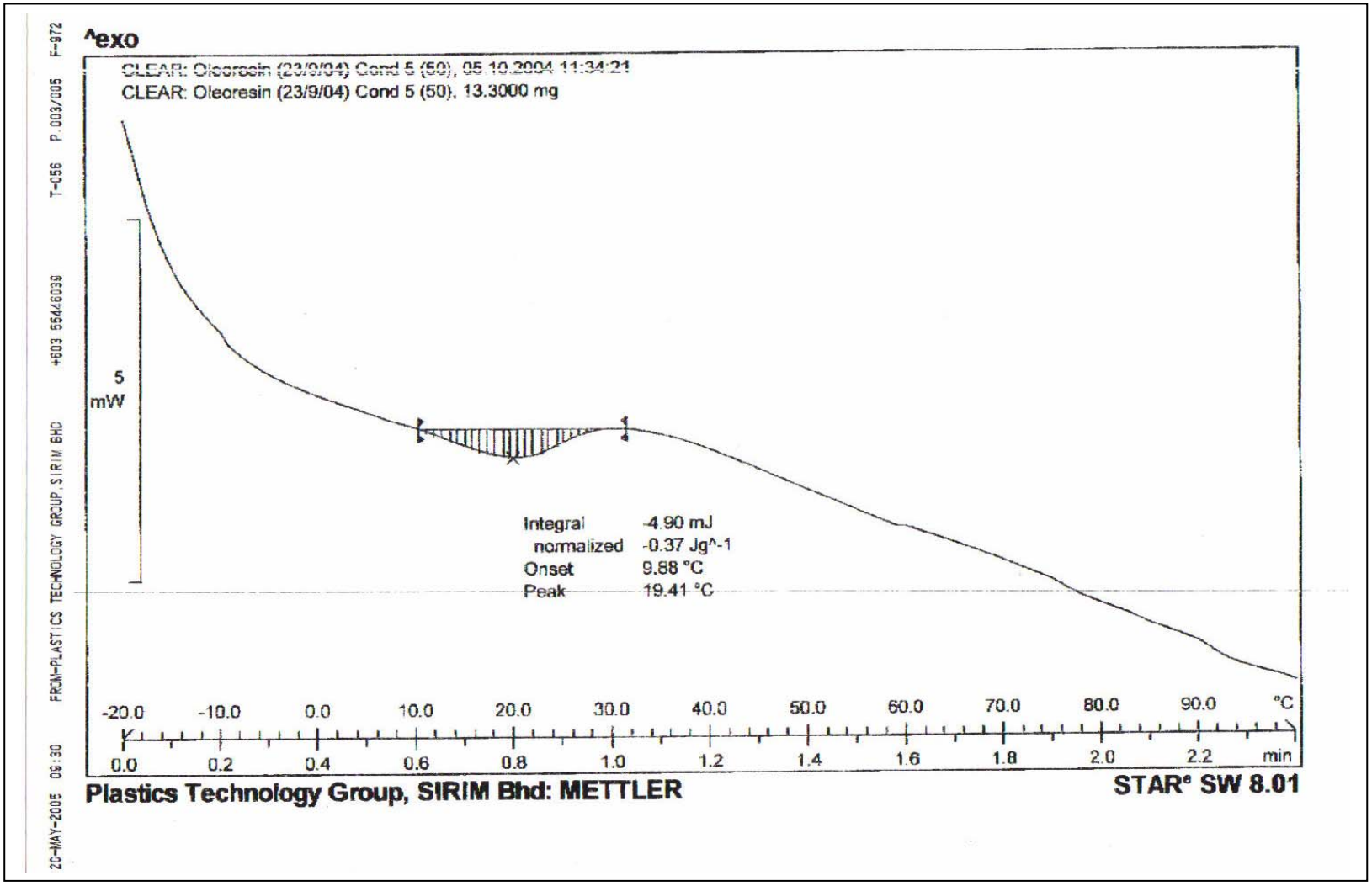
APPENDIX 7: GC-MS of compound isolated 6-gingerol)



APPENDIX 8: DSC thermogram of glycerol (ORIEM).



APPENDIX 9: DSC thermogram of ginger oleoresin (ORIEM).



APPENDIX 10: DSC thermogram of ginger oleoresin (SIRIM).

APPENDIX 11: Calculation of purity determination using DSC

Sample: Stearic acid, GC ~ 97%

T_0 represents the theoretical melting point of the pure substance [72] = 341.97 K

$T_m = T$ represent the melting point of impure sample (K)

$\Delta\overline{H}_{f,A}$ represents the enthalpy of fusion (J/mol)

} from DSC thermogram

R represents gas constant = 8.314 J/mol.K

x_A = mole % purity of sample

x_B = mole % impurity of sample

b) Glycerol: 50.000 ºC/min

T_0 = 303.15 K

T = 18.141 °C = 291.291 K

$\Delta\overline{H}_{f,A}$ = 210.6 mJ/mg x 284.49 g/mol =59913.59 J/mol

$$\ln x_A = \ln(1 - x_B) = \frac{\Delta\overline{H}_{f,A}}{R}\left(\frac{1}{T_0} - \frac{1}{T}\right)$$

$$\ln x_A = \frac{59913.59 J/mol}{8.314 J/mol.K}\left(\frac{1}{341.97 K} - \frac{1}{341.70 K}\right)$$

$x_A = e^{-0.0167}$

x_A = 98.35 mole % purity

APPENDIX 12: Calculation of purity determination using DSC.

Sample: **6-Gingerol**, HPLC ~ 98%

T_0 represents the theoretical melting point of the pure substance [72]

$T_m = T$ represent the melting point of impure sample (K)

$\Delta\overline{H}_{f,A}$ represents the enthalpy of fusion (J/mol)

} from DSC thermogram

R represents gas constant = 8.314 J/mol.K

x_A = mole % purity of sample

x_B = mole % impurity of sample

a) Scanning rate: 50.000 ºC/min

T_0 = 303.15 K

T = 28.930 °C = 302.080 K

$\Delta\overline{H}_{f,A}$ = 50.6 mJ/mg x 294 g/mol = 14876.4 J/mol

$$\ln x_A = \ln(1 - x_B) = \frac{\Delta\overline{H}_{f,A}}{R}\left(\frac{1}{T_0} - \frac{1}{T}\right)$$

$$\ln x_A = \frac{14876.4 J/mol}{8.314 J/mol.K}\left(\frac{1}{303.15K} - \frac{1}{302.08K}\right)$$

=1789.32(0.0032987-0.0033104)

=-0.020935044

$x_A = e^{-0.020935044}$

=0.979282573

x_A = 97.93 mole % purity

b) Glycerol: 50.000 ºC/min

T_0 = 303.15 K

T = 18.141 °C = 291.291 K

$\Delta\overline{H}_{f,A}$ = 210.6 mJ/mg x 284.49 g/mol =59913.59 J/mol

$$\ln x_A = \ln(1 - x_B) = \frac{\Delta\overline{H}_{f,A}}{R}\left(\frac{1}{T_0} - \frac{1}{T}\right)$$

$$\ln x_A = \frac{59913.59 J/mol}{8.314 J/mol.K}\left(\frac{1}{341.97K} - \frac{1}{341.70K}\right)$$

$x_A = e^{-0.0167}$

x_A = 98.35 mole % purity

area of the curve= mW *C
= mJ/s * C
= mJ *C/s * 1/Scanrate * 1/mass
=AH = mJ/mg