DHIYATUL RIDHWAN BACHELOR OF CHEMICAL ENGINERING (BIOTECHNOLOGY) 2013 UMP

POTENTIAL EVALUATION OIL PALM FROND JUICE FOR BIOETHANOL FERMENTATION

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POTENTIAL EVALUATION OIL PALM FROND JUICE FOR BIOETHANOL FERMENTATION

DHIYATUL RIDHWAN BIN MD NASARUDIN

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
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JULY 2013

SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology).

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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Dedication

To my supervisor and master student

ACKNOWLEDGEMENT

In the name of Allah S.W.T the Most Beneficent and the Most Merciful. The deepest sense of gratitude to the Almighty for the strength and ability to complete this project. Infinite thanks I brace upon Him.

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- Master student, Ms Norhazimah binti Abdul Halim for further guidance and giving a hand, especially in analysing process.
- Faculty of Chemical and Natural Resources for lending the laboratory for experimental process.

ABSTRACT

The fossil fuels degrade abundantly since 21st century. The usages of fossil fuels tend to emit the greenhouse gases that effect to global warming as well human health. To overcome these problems, the bioethanol introduced by the researches to substitute the fossil fuels. Bioethanol is a bioenergy produced by yeast fermentation at several conditions compared to conventional fossil fuels. Bioethanol is being studied because the unique properties and renewable fuels. The production of bioethanol in industrial scale involved high cost due to the usage of foods as sugar raw materials. Thus, this research is to evaluate the oil palm frond (OPF) juice as raw material for bioethanol fermentation. In this study, the bioethanol produces in shake flask by using *Saccharomyces cerevisiae* and OPF juice as medium component. Three parameters study are initial pH of medium, agitation speed, and OPF juice concentration. The best condition of parameters determine by the highest ethanol concentration produce. Inoculum prepared in shake flask to cultivate the yeast in medium growth and ferment in 250 ml shake flasks containing medium growth and beef extract. The flasks incubate in 48 hours, 12 hours interval for sample withdraw. The products analyse for sugar concentration and bioethanol concentration. The expected results for the best condition in bioethanol fermentation are in pH 7, 150 rpm agitation speed and 100% volume percentage of OPF juice concentration.

ABSTRAK

Bahan api fosil telah menyusut secara mendadak sejak abad ke-21. Penggunaan bahan api fosil menghasilkan gas-gas rumah hijau yang mengakibatkan pemanasan global dan juga menjejaskan kesihatan manusia. Untuk mengatasi masalah ini, bioetanol telah diperkenalkan oleh para pengkaji untuk menggantikan bahan api fosil. Bioetanol merupakan bio-tenaga yang dihasilkan melalui proses penapaian atas syarat-syarat tertentu berbanding dengan bahan api konvensional. Bioetanol dikaji atas sebab sifat-sifat yang unik dan bahan api yang boleh diperbaharui. Penghasilan bioetanol dalam skala industri melibatkan kos yang tinggi kerana penggunaan bahan-bahan makanan sebagai bahan mentah. Oleh demikian, kajian ini dijalankan untuk menilai pelepah kelapa sawit sebagai bahan mentah bagi proses penapaian bioetanol. Dalam kajian ini, bioetanol dihasilkan dalam kelalang kon menggunakan *Saccaromyces cerevisiae* dan jus pelepah kelapa sawit sebagai medium komponen. Tiga parameter yang dikaji ialah pH awal medium, kelajuan pengocakkan, dan kepekatan jus pelepah kelapa sawit. Keadaan parameter yang terbaik bergantung kepada berapa banyak kepekatan bioetanol yang dihasilkan. Inokulum disediakan di dalam kelalang untuk membiak dalam medium pembiakan dan diampai dalam 250 ml kelalang yang mengandungi medium pembiakan dan ekstrak daging. Kelalang-kelalang tersebut diperam selama 48 jam, di mana setiap 12 jam sampel diambil. Produk-produk ini menilai kehadiran kepekatan gula dan kepekatan bioetanol. Hasil yang dijangka bagi keadaan yang terbaik dalam penapaian bioetanol ialah dalam pH 7, 150 rpm dan 100% kepekatan jus pelepah kelapa sawit.

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LIST OF ABBREVIATIONS

µg/g	microgram per gram
µm	micrometre
%	percent
% v/v	percentage of volume over volume
^{o}C	degree Celsius
cm	centimetre
g	gram
g/g	gram over gram
g/h	gram per hour
g/l	gram per litre
J	Joule
K	Kelvin
kg	Kilogram
kPa	kilopascal
L	litre
M	molar
MJ	mega Joule
ml	millilitre
Nm	nanometre
Pa	Pascal
pH	Potential Hydrogen
ppm	part per million
rpm	rotate per minute
S	second
w/w	weight over weight

LIST OF ABBREVIATIONS

ANWR	Arctic National Wildlife Refuge
BTU	British Term Unit
CH_3CH_2OH	Ethanol
CO	Carbon monoxide
CO_2	Carbon dioxide
DOE	Design of Experiments
EtOH	Ethanol
EU	European Union
GC-FID	Gas Chromatography – Flame Ionization Detector
HCl	Hydrochloric acid
HPLC	High Pressure Liquid Chromatography
i.e.	*id est* (that it or in other word)
NaCl	Sodium chloride
NaOH	Sodium dioxide
OD	Optical Density
OPF	Oil Palm Frond
OPT	Oil Palm Trunk
Ppm	Part per million
Rpm	Rotate per minute
US	United States
YPD	Yeast-Peptone-Dextrose

1 INTRODUCTION

1.1 Motivation and statement of problem

The fossil fuels are non-renewable. They are limited in supply and will one day be depleted. There is no escaping this conclusion. Fossil fuels formed from plants and animals that lived hundreds of millions of years ago and became buried way underneath the Earth's surface where their remains collectively transformed into the combustible materials we use for fuel. For example, petroleum is the source of about 170 quadrillion BTUs or quad of energy of more than 460 quads the world uses, far more than derived from other sources (Zhang *et al.*, 2011).

The negative global warming impact of fossil fuels, volatile oil price and political unstable in oil exporting countries resulted in a significant increase in international interest in alternative fuels and led policy makers in the world to issue ambitious goals for substitution of alternative for convention fuels (Zhang *et al.*, 2011). Besides that, the great dangers posed to natural ecosystems that result from collecting fossil fuels, particularly coal and oil. Oil spills have devastated ecosystems and coal mining has stripped lands of their vitality. This is the primary reason to discontinue the pursuit to tap the vast oil reserves in the Arctic National Wildlife Refuge (ANWR).

In other to reduce the side effects of fossil fuels, the alternative energy is the best choice. One of the alternative energy is bioenergy, produced from the biological process. Examples of sources of bioenergy include wood and sawmill waste, charcoal, biogas resulting from the anaerobic decomposition of waste, as well as liquid biofuels, such as bioethanol and biodiesel. Bioethanol made biologically by fermentation from a variety of biomass sources is widely recognized as a unique transportation fuel and original material of various chemical with powerful economic, environmental and strategic attributes (Zhang *et al.*, 2011).

Nowadays, the studies of bioethanol is become interest in researchers. The production of fermentation by using sugar from sugar canes, grains, sweet potatoes and corn-starch are been commercialised. Since the sugar source is come from the food starch, the cost of the fermentation is high. The use of renewable resource such as agricultural and agro based-industry wastes as raw material for the fermentable sugars can help to reduce the production cost and the dependence on food crops (Zahari *et al.*, 2012). Thus, the raw material used in this study is wastes based from oil palm industry which is oil palm frond.

Oil palm contains abundant of sugar composition in all of the parts. Sulaiman *et al.* (2012) cited that the oil palm trunk (OPT) is contains higher sugar composition rather other part of oil palm. Thus, the OPT is the main raw material for ethanol fermentation. The OPT sap been hydrolysed to obtain sugar i.e. glucose and xylose (Yeoh *et al.*, 2001) but recently the sap is simply pressed (Kosugi *et al.*, 2010). This step is simple compared to OPT sap hydrolyse.

In addition, the composition of sugar in oil palm frond (OPF) is similar as the sugar composition in OPT. The production of sugar from dried OPF fibre involving the conversion of cellulose and hemicellulose into glucose and xylose through hydrothermal treatment followed by enzymatic hydrolysis, use of high temperature and pressure, and also cellulose enzymes. However, the renewable sugars can be obtained from OPF simply by pressing the fresh OPF to obtain the juice as cited by Zahari *et al.* (2012). To the best of our knowledge, there is no research has been done by any researcher to optimize the bioethanol production from OPF juice especially using statistically design of experiment (DOE).

1.2 Objectives

The aim of this research is to produce higher production of bioethanol by evaluate the potential of bioethanol production from OPF juice. The objectives are:

(i) To study the effect of process parameters (pH, agitation speed and OPF juice concentration) for the production of bioethanol from fermentation using OPF juice

(ii) To evaluate the best condition for the production of bioethanol in term of yield and productivity from fermentation using OPF juice as renewable and sustainable substrate.

1.3 Scope of this research

In order to achieve the stated objectives, the following scopes have been identified:

i) To evaluate the feasibility of OPF juice as a direct substrate for bioethanol production by using yeast *Saccharomyces cerevisiae.*

ii) Three main factor affecting bioethanol productions from literature studies are using lab scale experiment. Experiments are carried out as a function of initial pH, agitation rate, and percentage of OPF juice.

iii) To identify the ethanol yield and productivity of each parameter given.

1.4 Main contribution of this work

The significant of study obtained by researchers is reducing the high cost of raw material used. This is because, currently ethanol are produced from food as raw material such as sugar canes, corns, sweet potatoes and cassava. Therefore, by using biomass from oil palm can reduced the production cost as well as dependence on the food corps.

Then, the usage of bioethanol as new potential source compared to fossil fuel can reduced the amount of carbon dioxide (CO_2), carbon monoxide (CO), hydrocarbon and particulate matter with aid of oxygen and no aromatics compound such as sulphur content emitted into atmosphere. Thus, the greenhouse effect also been reduced.

The research also tends to optimise all part of the oil palm biomass. This is very important because it can help to reduce the amount of biomass generated from palm oil industry. The OPF also appropriate as sugar raw material in similar fermentation, as using empty fruit bunches.

1.5 Organisation of this thesis

The structure of the reminder of the thesis is outlined as follow:

Chapter 2 provides a description the bioethanol, its properties, world production, market demand, as well as production method. The description of oil palm, its biomass production involves the increasing of waste product, and importance of the OPF consisting of the sugar composition as suitable raw material for ethanol fermentation.

Chapter 3 gives a review of the experimental design of the research, beginning from the OPF juice preparation, followed by fermentation of OPF juice depending to the parameter setting. The sample taken each 12 hour interval for 48 hours. The sample then analyse by using gas chromatography (GC-FID) for bioethanol determination.

Chapter 4 is devoted to a highest production of the bioethanol based on the parameter setting. The peak area of the sample recorded from CG-FID converted into the product concentration via the standard curve of bioethanol prepared. In this chapter also discuss about the effect of the parameter used in bioethanol fermentation.

Chapter 5 draws together a summary of the thesis and outlines the future work which might be derived from the potential of bioethanol production.

2 LITERATURE REVIEW

2.1 Overview

This chapter discussed about the bioethanol and its properties. This included the world production of the bioethanol, the market demand, and method used in order to produce bioethanol. This also discussing about oil palm and biomass that produced from oil palm. Based from the biomass discussed, the oil palm frond has introduce. The sugar composition from the oil palm frond is found and available as raw material for the bioethanol production.

2.2 Bioethanol

Bioethanol (ethyl alcohol, grain alcohol, CH_3-CH_2-OH or EtOH) is a liquid biofuel which can be produced from several different biomass feed stocks and conversion technologies (Balat *et al.*, 2008). Bioethanol is an attractive alternative because it is a renewable bio based resource and it is oxygenated thereby provides the potential to reduce particulate emissions in comparison ignition engines. The properties of ethanol are shown in Table 2.1.

Table 2.1: Characteristics and properties of ethanol

Molecular formula	Units	Values
Molar mass	g/mol	46.07
Density	g/cm	0.789
Melting point	^oC	-114
Boiling point	^oC	78.37
Vapour pressure	kPa (at 20^oC)	5.59
Acidity (pK_a)	-	15.9
Basicity (pK_b)	-	-1.9
Refractive index (n_D)	-	1.36
Viscosity	Pa.s (at 20^oC)	0.0012
Dipole moment	-	1.69 D

Bioethanol has a higher octane number, broader flammability limits, higher flame speeds and higher heats of vaporisation than gasoline. Bioethanol also possesses a high octane fuel, subsequently has replace lead as octane enhancer in petrol (Taherzadeh, 1999). Some of properties of alcohol are shown in Table 2.2 (Balat *et al.*, 2008).

Table 2.2: Some properties of alcohol fuels

Fuel property	Isoctane	Methanol	Ethanol
Centane number	-	5	8
Octane number	100	112	107
Auto-ignition temperature (K)	530	737	606
Latent heat of vaporisation (MJ/kg)	0.26	1.18	0.91
Lower heating value (MJ/kg)	44.4	19.9	26.7

Bioethanol is widely used for strong alcoholic drinks production and to less degree for medical or pharmaceutical purposes. Nowadays it also represents an important, renewable liquid fuel for motor vehicles and in mixtures with gasses, substitute for natural gas (Ranković *et al.*, 2009). Cheng *et al.* (2002) mention the bioethanol is one form of renewable energy source that is fast gaining foothold as potential fuel of power automotive engine.

2.1.1 World Production of Bioethanol

Bioethanol is the most widely used liquid biofuel. The largest producers in the world are United States, Brazil, and China. Production of bioethanol from sugar cane in Brazil accounted nearly 18% of the country's automotive fuel needs in 2004 (Petrova and Ivanova).

In 2006, global production of bioethanol reached 13.5 billion gallons up to from 12.1 billion gallons in 2005 (Balat *et al.* 2008). World ethanol production (all grades) has been reached a record 62×10^9 litre in 2007, with the United States and Brazil as dominant producers (approximately 70%) (Licht, 2008).

In 2009, the US produced 39.5×10^9 litres of ethanol using corn as a feedstock while the Brazil created about 30×10^9 litres of ethanol using sugar cane (Petrova and Ivanova, 2010). Balat *et al.* (2008) stated that 90% of bioethanol is derived from corn in United States and all the Brazil's bioethanol is produced from sugar cane. The top ten bioethanol produces are presented in Table 2.3.

Table 2.3: The top ten bioethanol producers (billion gallons)

Country	2004	2005	2006
USA	3.54	4.26	4.85
Brazil	3.99	4.23	4.49
China	0.96	1.00	1.02
India	0.46	0.45	0.50
France	0.22	0.24	0.25
Germany	0.07	0.11	0.20
Russia	0.20	0.20	0.17
Canada	0.06	0.06	0.15
South Africa	0.11	0.10	0.10
Thailand	0.07	0.08	0.09

Balat *et al.* (2008) cited that any country with a significant agronomic based economy can used current technology for bioethanol fermentation due to technology for bioethanol production from non-food plant sources has been developed to the point at which large scale production will be reality in the next few years (Lin *et al.*, 2006).

2.1.2 Market Demand

The potential bioethanol production could replace 353 billion litres of gasoline (32% of the global gasoline consumption) when bioethanol is used in E85 fuel for a midsize passenger vehicle (Balat *et al.*, 2008). The demand for bioethanol is expected to increase dramatically until 2020. The US oil imports will be reduced by nearly 4 billion barrels over that time since the US signed an executive order specifying a tripling in the production of bio based products and bioenergy by the year 2010 (Patrova and Ivanova, 2010).

Efforts to decrease greenhouse gas emissions are expected to spur the production of renewable energy sources by 6% within the European Union by 2010 (Zaldivar *et al.*, 2001). In France, the approval of a clean air act could increase ethanol production to 500 million litres. Similar projects in Spain, Sweden and Netherlands are expected to increase the utilization of ethanol to account for 15% of transportation fuels by 2010 (Mansson and Foo, 1998). The EU market for fuel ethanol will grow considerably in coming years, as a result of the EU policy to substitute 8% of fossil transport fuels by renewable biofuels by the year 2020. Balat *et al.* (2008) cited that the potential demand for bioethanol as fuel for transportation in EU countries, calculated on the basis of

Directive 2003/30/EC, is estimated at about 6 billion litres in 2006 and 12.7 billion litres in 2010.

Meanwhile in Brazil, most of the bioethanol is used domestically substituting 40% of Brazilian petrol consumption and approximately 20% is exported to the United States, EU and other markets (Balat *et al.*, 2008).

2.1.3 Method Production

Typically, researches on the method production are depending on the availability of raw material in that used. Table 2.4 showed the researches on bioethanol from the different material.

Table 2.4: Research of bioethanol from different raw material

Raw material/carbon source	Pre-treatment	Strain	Maximum yield of ethanol (g/l)	Time (h)	Reference
Cow's whole milk	Boiled for 20 minutes and cooled, followed by filtering and sterilised	*Kluyveromyces marxianus* strain NCIM 3551	19	32	Gupte and Nair (2010)
Empty fruit bunches	Dilute alkaline pre-treatment, followed by mild acid hydrolysis and enzymatic saccharafication	*Saccharomyces cerevisiae* strain ATCC 24860	50	72	Kassim *et al.* (2011)
OPT sap	No	*Saccharomyces cerevisiae* Kyokai no. 7	30	48	Kosugi *et al.* (2010)
Sweet potato	Mash the potato and liquefaction	*Saccharomyces cerevisiae* strain CCTC M206111	128.51	30	Zhang *et al.* (2011)
Spirogyra	Washed with water and dried for three days. Mashed with 5:1 aquadest with aquadest in Spirogyra, sterilised and hydrolysed	*Saccharomyces cerevisiae* and *Zymomonas mobilis*	9.70	96	Sulfahri *et al.* (2011)
Ziziphus mauritiana	6 days drying and gently crushed	*Saccharomyces cerevisiae* strain NA33	70	168	Togarepi *et al.* (2012)

Refer to Table 2.4, the raw material that used are cow's whole milk (Gupte and Nair, 2010), empty fruit bunches (Kassim *et al.*, 2011), OPT sap (Kosugi *et al.*, 2010), sweet potato (Zhang *et al.*, 2011), Spirogyra (Sulfahri *et al.*, 2011) and *Ziziphus mauritiania* (Togarepi *et al.*, 2012). However, various pre-treatments such as mechanical and steam pre-treatments are needed in order to loosen up the lignocellulosic structure (Zahari *et al.*, 2012). All the research above used in batch system.

Based on Table 2.4 also, the research used similar yeast yet differs in strain types i.e. *S. cerevisiae* strain ATCC 24860 (Kassim *et al.*, 2011), Kyokai no. 7 (Kosugi *et al.*, 2010), strain CCTC M206111 (Zhang *et al.*, 2011) and *S. cerevisiae* strain NA33 (Togarepi *et al.*, 2012). Moreover the other research used the different yeast or microorganism for bioethanol production i.e. *Kluyveromyces marxianus* strain NCIM 3551 (Gupte and Nair, 2010), and *Zymomonas mobilis* (Sulfahri *et al.*, 2011). Most of the yeast cells used suffer from various stresses, including environmental stress during ethanol fermentation such as glucose concentration, nutrient deficiency, temperature, rate of agitation and pH (Graves *et al.*, 2006; Arisra *et al.*, 2008; Yah *et al.*, 2010).

2.3 Oil Palm

The oil palm tree (*Elaeis guineensis*) is indigenous to the tropical forest in West Africa. The oil palm tree has become one of most valuable commercial cash corps in Malaysia, due to increase from 3.37×10^6 hectares in 2000 to 4.50×10^6 hectares in 2005. In 2010, 4.85 million hectares planted in Malaysia. Thus, the amount of planting the oil is creating a significant biomass that can be converted into an add value products (Sulaiman *et al.*, 2012).

The development of the oil palm plantation in Malaysia had grown increasingly up until the end of the twentieth century. Malaysia and Indonesia have become dominant in the trade and have begun producing a great deal of palm oil and palm kernel oil in this century. Almost 80% of the world oil palm plantation centres in Southeast Asia. Most of plantation occurring in Malaysia and Indonesia, 260,000 hectares planted in Thailand and the rest in the Philippines, Cambodia and Myanmar (Sulaiman *et al.*, 2012). The plantation area in Malaysia and Indonesia in 2007 was 4,304,913 hectares, and nearly 7 million hectares respectively (Kosugi *et al.*, 2010).

Total exports of oil palm products increased from 22.43 million tons in 2009 to 23.06 million tons in 2010 (Sulaiman *et al.*, 2012). Meanwhile, Kosugi *et al.* (2010) stated that the combined palm oil production in Malaysia and Indonesia accounts for approximately 88% of the worldwide production.

The factors that led to expansion of the harvesting area in Southeast Asia are the increasing in the consumption of dietary oils and fats in China and India, as well as strengthened the market price of palm oil and kernel oil, tend to encourage investors to develop plantations in the large areas of suitable land found in Peninsular Malaysia, Islands of Sumatra and Borneo (Sulaiman *et al.*, 2012).

Figure 2.1: Oil palm tree

2.4 Biomass

Biomass refers to any organic plant product that has general uses. The amount of biomass produces is 30 million tonnes in empty fruit bunches (EFB), oil palm trunks, and oil palm fronds. The trunks and fronds are obtained from palm growing and plantation, meanwhile empty fruit bunches, fibre, shell, and effluent are obtained from oil palm processing (Sulaiman *et al.*, 2012).

Malaysia produces a large amount of residues due to increasing global demand for palm oil. The abundant oil palm biomass will produce. The research on oil palm biomass has begun to help reduce oil palm biomass waste and at the same time, increase the economic return to the country. The oil palm industry produces 85.5% of biomass in Malaysia (Sulaiman *et al.*, 2012) widely used for industrial purposes, foods, and recently has been reconsidered as a material for the production of biodiesel and bio plastics (Kosugi *et al.*, 2012).

Kassim *et al.* (2011) mentioned that biomass can be varied promising alternative source of renewable energy. The utilization of biomass also offers an environmentally friendly way of disposing the unwanted by-products generated from oil palm mills.

2.5 Oil Palm Frond

Oil palm (*Elaeis guineensis*) for palm oil production needs to be replanted at an interval of 20 to 25 years in order to maintain oil productivity (Kosugi *et al.*, 2010). Sulaiman *et al.* (2012) states that the oil palm fronds are available when the economic lifespan of the palm reaches at time replanting. The average age of replanting is 25 years approximately.

Zahari *et al.* (2012) cited that the most generated oil palm biomass is oil palm frond (OPF), which amounted to 83 million tonnes (wet weight) annually. OPF is obtained during pruning for harvesting fresh fruit (FFB), therefore it is available daily.

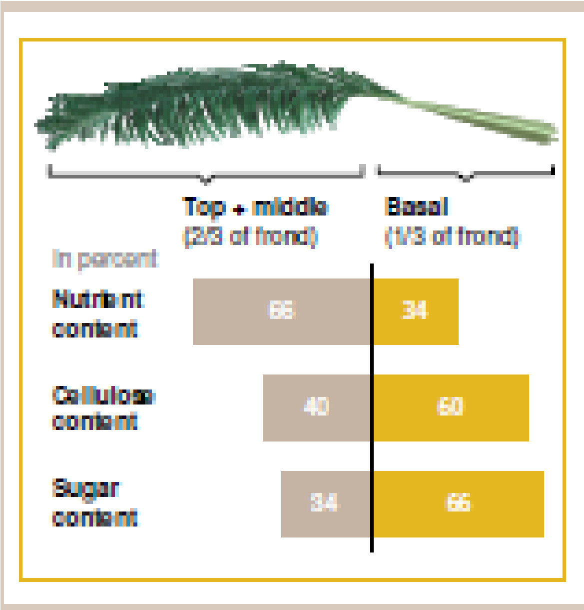

Figure 2.2: Oil palm frond juice and its composition (National Biomass Strategy 2020, 2011)

2.6 Chemical Composition in Oil Palm Frond

The OPF contains high dietary fibre (21%) and carbohydrate (28%), with low crude protein of 3.2% (Zahari *et al.*, 2012). The cellulose, hemi-cellulose, and lignin contents of the fresh OPF in this study were 41.7%, 16.4% and 15.5%, respectively. Since the fresh OPF has high holocellulose content, the potential amount of renewable sugars from OPF can be increased if saccharification is done to further enhance the sugar yield from the fibre.

Table 2.5 shows the nutrients and metallic elements in the OPF and OPF juice. The OPF and OPF juice contained high percentage of carbon, i.e. 49% and 39% respectively. The high carbon content in the OPF juice indicates its suitability as a renewable carbon source for the production of value-added product such as bioethanol, lactic acid and biobutanol through bacterial fermentation. In addition, the metal concentration in the OPF and OPF juice also shown in Table 2.5. The result shows low heavy metals concentration (<100 ppm) in the OPF juice, showing its suitability as fermentation feedstock.

Table 2.5: Nutrient and metallic elements in OPF and OPF juice

Analysis	Fresh OPF	OPF juice
N (%)	0.9	0.8
C (%)	49	39
#OC (%)	37	29
Composition of nutrients and metal elements		
S (%)	0.2	0.4
P (%)	0.02	0.02
K (%)	0.2	2.3
Ca (%)	1.4	2.9
Mg (%)	0.2	0.5
B (ppm)	4	2
Mn (ppm)	61	2
Cu (ppm)	2	2
Fe (ppm)	100	66
Zn (ppm)	3	9

Organic carbon
Data is the mean of duplicate samples

Table 2.6 shows the comparison of sugar concentration between OPF juice (Zahari *et al.*, 2012) and oil palm trunk (OPT) sap (Kosugi *et al.* 2010). As shown in Table 2.6, the amount of sugars in OPF juice is 76.09±2.85 g/l. Glucose was the major sugar component in OPF juice at 53.59±2.86 g/l, sucrose at 20.46±1.56 g/l and fructose at 1.68±0.75 g/l. Meanwhile, the sugar composition in OPT sap is 59.5±5.6 g/l. Similar to OPF juice, glucose was the major component in OPT sap at 50.2±2.8 g/l, followed by sucrose at 3.8±0.5 g/l and fructose at 3.1±1.3 g/l and the other sugar composition shown in Table 2.6. The presence of sugar in OPF and OPT was expected due to the photosynthesis process.

Table 2.6: Composition of sugar in OPF juice and OPT sap

Sugar	OPF juice	OPT sap#
Fructose (g/l)	1.68±0.75	3.1±1.3
Glucose (g/l)	53.95±2.86	50.2±2.8
Sucrose (g/l)	20.46±1.56	3.8±0.5
Xylose (g/l)	-	1.0±0.4
Galactose (g/l)	-	0.9±0.4
Rhamnose (g/l)	-	0.5±0.2
Total (g/l)	76.09±2.85	59.5±5.6

Average of sugars composition in OPT sap

The total amino acids in OPF juice were 17.41 µg/g, with serine (111.0 µg/g), glutamic acid (22.7 µg/g) and proline (27.1 µg/g) as major amino acids. The amino acid composition is almost similar to that of OPT sap reported by Kosugi *et al.* (2010).

2.7 *Summary*

As conclusion, the bioethanol is sustainable for fossil fuel replacement due to the properties and renewable energy. The world production of bioethanol held in American region and market demand in both American region and Europe this the proof for the availability of bioethanol usage especially as fuel. Therefore, the research held the research of bioethanol by using food as a raw material. However, this raw material tend to increase the cost of fermentation. Thus, the researcher introduce the biomass as raw material. The oil palm produce abundant of biomass such as EFB, OPT, OPF and so on. Since the OPF not expose yet as raw material, the previous researcher held the experiment to verify the sugar composition in OPF. Zahari *et al.* (2010) proof the presence of the sugar composition in OPF juice and the composition is slightly similar with OPT sap. So, the OPF is sustainable as raw material for bioethanol production.

3 MATERIALS AND METHODS

3.1 Overview

This chapter discusses more about the methods and procedures imply in all experiments including preparation of medium, reagent and also analytical method. The experiment is divided into three major parts, which are the effect of pH study, the effect of agitation speed study and the effect of oil palm frond juice study. The fermentation of oil palm frond juice by yeast strain studies in incubator shaker. All the fermentation conducts in a 250 ml shake flask seal with a rubber stopper and a one-way air valve before purging with nitrogen gas to ensure the anaerobic conditions in a flask maintain. Figure 3.1 shows the overall process in this research.

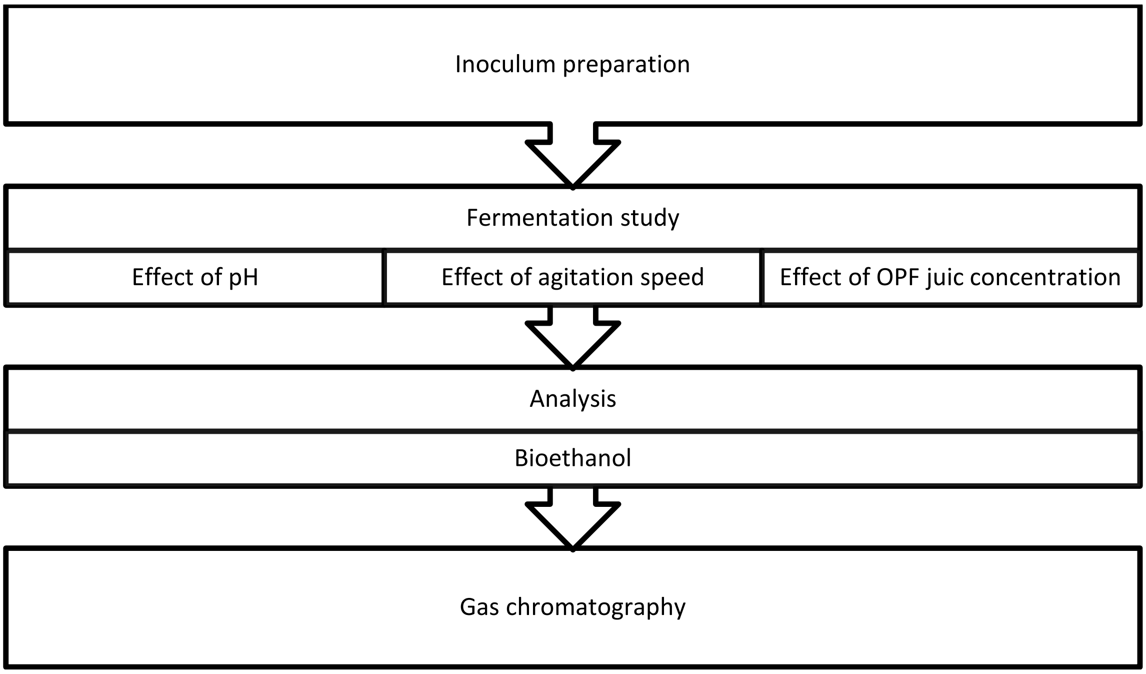

Figure 3.1: Overall process of bioethanol fermentation from OPF juice by S. cerevisiae Kyokai no. 7

3.2 Chemicals and Reagents

All the chemicals used in this study were analytical grade except for HPLC and GC-FID measurement (HPLC grade). Medium agar and nutrient were purchased from Sigma-Aldrich (Malaysia) whereas standard sugar was obtained from Daejung Chemicals (Taiwan).

3.3 Microorganism

The strain employed throughout these studies is an industrial sake brewing strain, *Saccharomyces cerevisiae* Kyokai no. 7 (ATCC 26422) obtained from the American Type Culture Collection. The strain is maintained by regular sub culturing on YPD agar medium (in g/l: agar 20, yeast extracts 1.5, peptone 1.5 and dextrose 20) every five weeks.

3.4 Inoculum Preparation

Inoculums of yeast strain *S. cerevisiae* Kyokai no. 7 (ATCC 26422) is prepared in 500 ml flask containing 250 ml of medium (enriched with NaCl 5 g/l, peptone 5 g/l, yeast extracts 1.5 g/l) and vented with cotton wool to allow aeration. Flasks are incubated for 24 hours at 30 ^{O}C and 150 rpm. This inoculums preparation applied in all parts of the experiment.

3.5 Production Medium

The production medium composition use in fermentation by using *S. cerevisiae* as microorganism and OPF juice as substrate are sodium chloride (NaCl) 5 g/l, peptone 5 g/l, beef extracts 1.5 g/l and yeast extracts 1.5 g/l. This medium inserts 100 ml into 250 ml Erlenmeyer flask.

3.6 Instrumentation Setup

Gas chromatography Agilent Technologies (6890 Series) equipped with a flame ionization detector (FID) was set up to determine ethanol concentrations. The column used was HP-INNOWax Polyethylene Glycol (30 m x 250 µm x 0.25 µm nominal). The oven temperature and injector and detector temperature are 70 °C and 250 ^{o}C respectively. Initial temperature 70 °C held for 4 min, rate of increase 20 °C/min until 120 °C min held for 2 min). The carrier gas was helium at a flow rate of 45 ml/min.

3.7 Experimental Design

3.6.1 Fermentation of OPF Juice by Yeast S. cerevisiae

Flask containing 100 ml of total working volume, including 10 %v/v of inoculum is incubated at 30^{o}C and agitation rate 150 rpm to check the ability of yeast to convert OPF juice to bioethanol. Temperature and agitation rate control using incubator shaker. The incubation carries out 48 hour. Samples are withdrawn aseptically at 12 hour intervals to measure ethanol yield and total sugar. The medium of OPF juice before and after fermentation were compared to check for existence of ethanol compound.

3.6.2 Effect on Initial pH

The first series of experiment is to determine the effect of initial pH on ethanol production. The range of pH used is pH 4, 6, 7, and 8. Initially, the pH of OPF juice adjusts using 1M hydrochloric acid (HCl) or 1M sodium hydroxide (NaOH) prior to inoculate with standardized *S. cerevisiae*. The samples incubated at 30^{o}C with an agitation speed of 180 rpm, OPF juice of 50%, and pH 7.

3.6.3 Effect on Agitation Speed

The second series of experiment is to determine the effect of agitation speed on ethanol production. The OPF juice incubate in different rates of agitation, i.e., 150 rpm, 180 rpm and 210 rpm at 50% of OPF juice, pH 7 and temperature of 30^{o}C.

3.6.4 Effect of OPF Juice Concentration

The third series of experiment is to determine the effect of OPF juice concentration on ethanol production. The OPF juice incubate in different concentration, i.e. 25%, 50% and 100% of OPF juice at agitation speed of 180 rpm, pH 7 and temperature of 30^{o}C. The parameters to be studied are summarised in Table 3.1.

Table 3.1: Parameter that to be studied in this research.

Parameter	Parameter Value
pH	4
	6
	7
	8
Agitation speed (rpm)	150
	180
	210
OPF juice concentration (v/v percentage)	25
	50
	100

3.8 Products Analysis

Internal and External Standard Preparation

n-propanol (1 %v/v) has been used as solvent or internal standard in all samples. Internal standard is being used as a reference standard for normalization of ethanol peak and also to mark the peak of ethanol in the chromatogram by relative retention time. It is previously diluted from 99.8 %v/v of HPLC grade *n*-propanol. For external standard, ethanol prepares in 5-30 g/l of concentration.

GC- FID Sample Preparation

Samples are prepared in the vial by filtering with 0.20 μm nylon filter. Peak of ethanol observed at R_t =2.985 min convert to the concentration term (g/l) using previous obtained standard curve.

3.9 Summary

This chapter present about the method and procedure implies in the bioethanol fermentation. The method implies in this fermentation process including the inoculum process for *Saccharomyces cerevisiae* Kyokai no. 7, the fermentation of OPF juice concentration according to the parameter used and the sample taken each 12 hour intervals for 48 hours fermentation. The sample then analyse by using GC-FID to determine the presence of bioethanol for each intervals. In this chapter also describe about the chemical and reagent, the microorganism, the inoculum preparation, production medium and instrumentation setup used in bioethanol production.

4 RESULT AND DISCUSSION

4.1 Overview

This chapter shows the result obtain from the experimental design in previous chapter. In this chapter, the standard curve of bioethanol production introduced to determine the bioethanol from the sample taken. The effect of each parameters shows in form of tabulated data and graph for trending of each parameters, as well as briefly explanation and discussion for each trend in parameters that study.

4.2 Standard Curve of Bioethanol

The standard bioethanol curve is derived from the standard bioethanol that concentrated from 5 to 30 g/l. The standard curve consist of the correlation between peak area of bioethanol detected from GC-FID and the concentration of the standard bioethanol via graphic method. Table 4.1 shows the peak area and the concentration of standard bioethanol prepared while Figure 4.1 show the relationship between peak area and the concentration of bioethanol prepared.

Table 4.1: Standard concentration of bioethanol and the peak area of bioethanol after analysing by GC-FID.

Bioethanol (g/l)	Peak Area (pA*s)
5	222
10	429
15	641
20	847
25	1047
30	1283

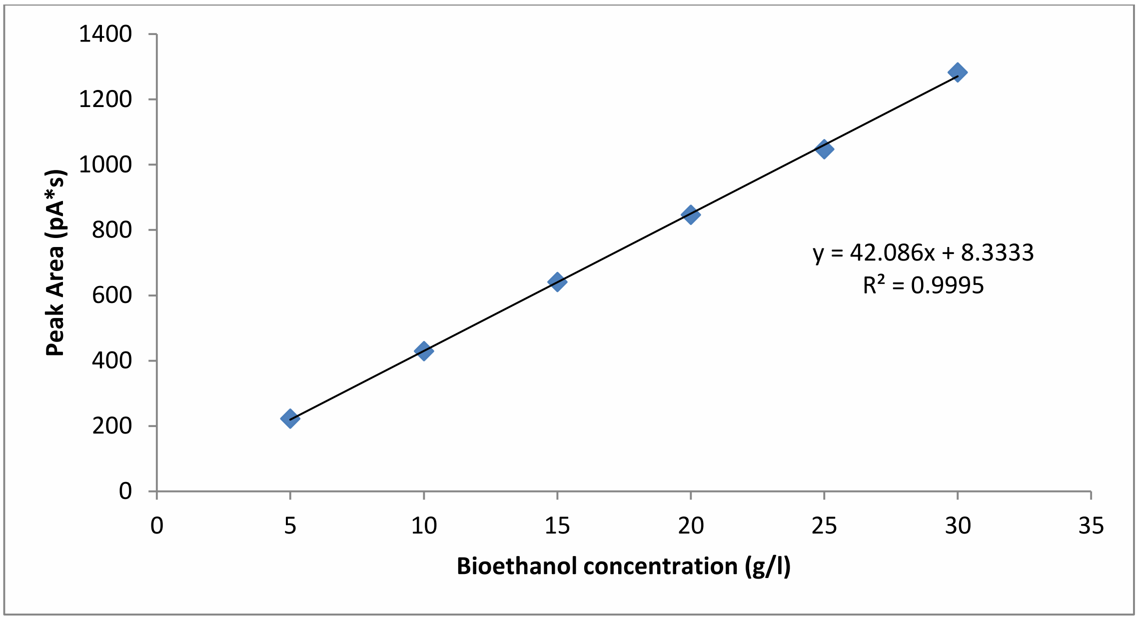

Figure 4.1: The correlation between the standard bioethanol concentration and the peak area.

Since the graph is precise and hit all the point, thus there is almost no error in this correlation. Therefore, the concentration of bioethanol in the sample also will precise as well.

This correlation meant for all the effect of the parameters that studied due to the bioethanol that detected from GC-FID used in term of peak area. Thus, the correlation able to applied in the bioethanol production to determine the concentration of bioethanol present in the sample for each effect of parameters.

4.3 Effect on Initial pH

The effect of initial pH has been reported to show a significant influence on fermentation, mainly on yeast growth, fermentation rate and by-product formation reported by Caudhary and Qazi (2006), Sheela *et al.* (2008), and Mandikandan *et al.* (2008). Thus, the initial pH setting as pH 4, 6, 7 and 8 via HCl and NaOH for both medium and OPF juice. The bioethanol concentration determine for all samples collected at 12 hour interval for 48 hours. Table 4.2 shows the bioethanol concentration for each sample collected and Figure 4.2 shows the bioethanol profile for the effect of initial pH.

Table 4.2: Effect on initial pH of medium on bioethanol production.

Time (h)	Bioethanol concentration (g/l)			
	pH 4	pH 6	pH 7	pH 8
0	0.147	2.473	0.427	0.425
12	2.290	1.843	6.267	4.901
24	8.339	3.680	24.097	12.932
36	22.185	3.799	29.596	17.832
48	24.922	4.996	29.665	18.530

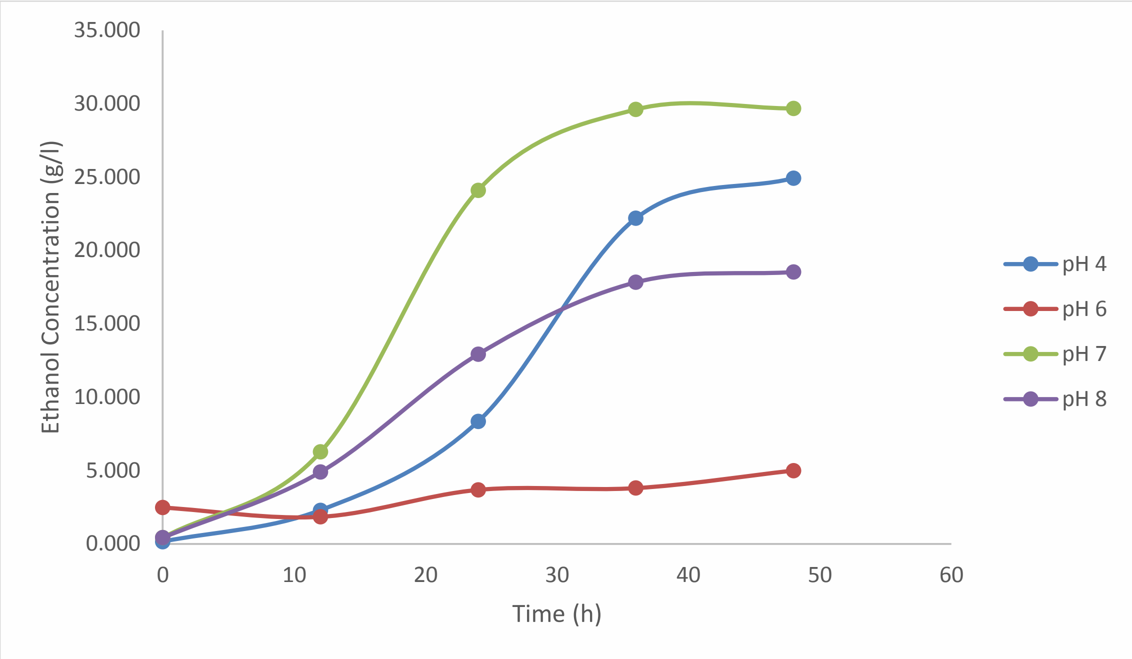

Figure 4.2: Bioethanol profile for the effect of initial pH medium.

The highest bioethanol production for the effect of initial pH medium is in pH 7, up to 29.67 g/l followed by pH 4, pH 8 and pH 6 which are 24.92 g/l, 18.53 g/l and 4.996 g/l respectively. The result is supported with other studies reported that yeast grows and fermentation process best in natural or slightly acidic environment (Noor *et al.*, 2005; Manikandan *et al.*, 2008).

Kassim *et al.* (2010) mention that the most suitable initial pH for bioethanol production is in pH 4. This statement supported by other studies that mentioned the highest bioethanol production is in pH 4 for initial pH of medium (Periyasamay *et al.*, 2009; Satyanagalakshmi *et al.*, 2011; Vaithanomsat *et al.*, 2009). According to Buzas *et al.* (1989), the optimum pH value for *S. cerevisiae* is in range of 4.2 to 4.8. However, this statement is contradict with the result shown due to indifference between the fermentation process conducted at pH 5.4 and pH 4.7 (Putri Faridatul and Puad, 1991) supported by

Wilkinson and Rose (1963) stated insignificant difference between fermentation yields conducted in pH range between 2.4 to 8.6.

The bioethanol production high when the pH value is lower. The lower bioethanol productivity may be due to the lower metabolic rate of cell yeast used (Mariam *et al.*, 2009). According to Munene *et al.* (2002), increasing of pH value in the molasses hydrolysate will reduce alcohol hydrogenase activity in *S. crevisiae* cell and lead to the formation of acidic acid instead of bioethanol in the process. The different strain also contribute the optimum pH for bioethanol production.

4.4 Effect on Agitation Speed

Mechanical agitation also plays an important role for bioethanol production, yeast cell viability and uniform mixing cell and nutrient within the medium components during the fermentation (Boswell *et al.*, 2002; Noor *et al.*, 2003; Arisra *et al.*, 2008). The agitation speed is setting to 150 rpm, 180 rpm and 210 rpm. Table 4.3 shows the bioethanol from each sample in 48 hours at 12 hour interval and Figure 4.3 illustrate the bioethanol profile for each value of agitation speed.

Table 4.3: Effect on agitation speed on bioethanol production.

Time (h)	Bioethanol concentration (g/l)		
	rpm 150	rpm 180	rpm 210
0	1.584	1.228	1.603
12	18.587	5.771	2.791
24	24.632	12.842	19.212
36	27.792	19.495	17.737
48	25.654	19.735	19.348

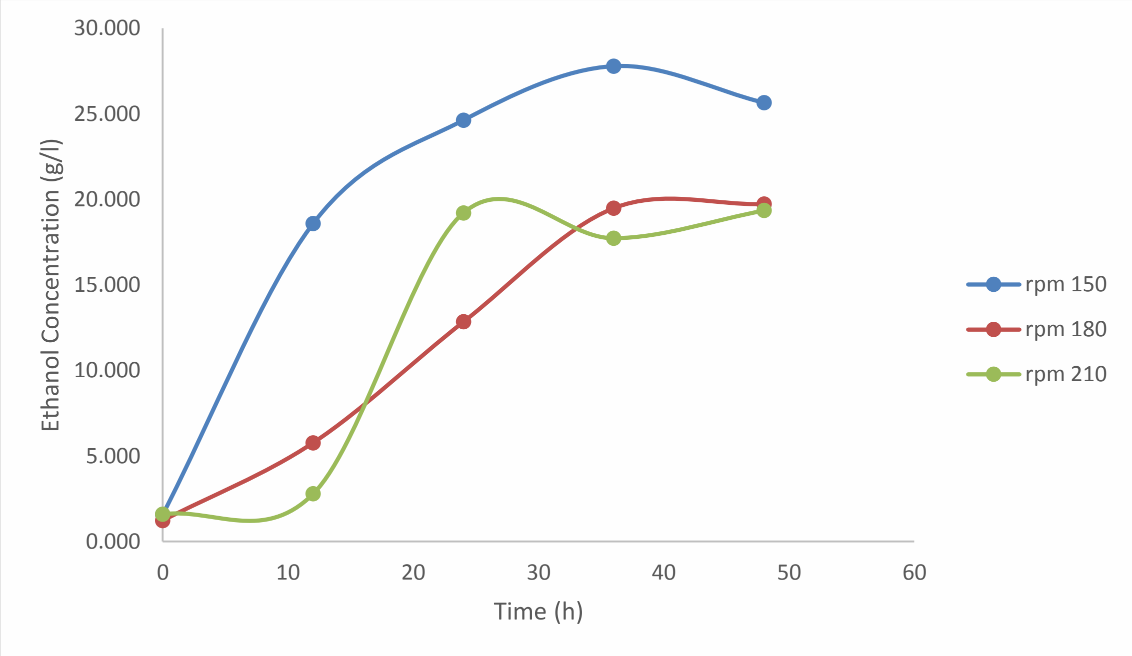

Figure 4.3: Bioethanol profile for the effect of agitational speed.

The highest bioethanol production is 27.79 g/l in 150 rpm at interval of 36 hour, followed by 180 rpm and 210 rpm which are 19.74 g/l and 19.21 g/l at interval of 24 hour respectively. Kassim *et al.* (2010) studied the effect of the agitation speed from 50 rpm to 150 rpm. Thus, this statement is in agreement with Noor and Hameed (1999) cited the bioethanol production yield using mechanical agitation was higher than without or lower agitation speed. However, this study consist the agitation speed above 150 rpm. Since the OPF juice concentration is constant, the viscosity of medium is constant and unaffected to bioethanol production. So, the increasing of agitation speed above to 150 rpm reduced the bioethanol production due to the turbulence of medium will create the shear force that cause the cells break down. Stountenburg *et al.* (2008) mentioned for the batch fermentation of wood hydrolysate by *Pichia stipitis* indicated the highest bioethanol production was obtain in 150 rpm with 0.37 g/g glucose after 72 hours fermentation.

4.5 Effect on OPF Concentration

The OPF concentration also contribute an important role for bioethanol production. Thus the OPF concentration is setting to the % v/v of 25, 50 and 100. Table 4.4 shows the bioethanol concentration from each sample in 48hours at interval of 12 hour and Figure 4.4 illustrate the bioethanol profile for each value of OPF concentration.

Table 4.4: Effect on OPF juice concentration (% v/v) for bioethanol production.

Time (h)	Bioethanol concentration (g/l)		
	25%	50%	100%
0	0.275	0.215	0.569
12	3.221	7.329	5.982
24	10.722	17.333	21.420
36	7.470	26.319	45.363
48	12.017	27.312	41.307

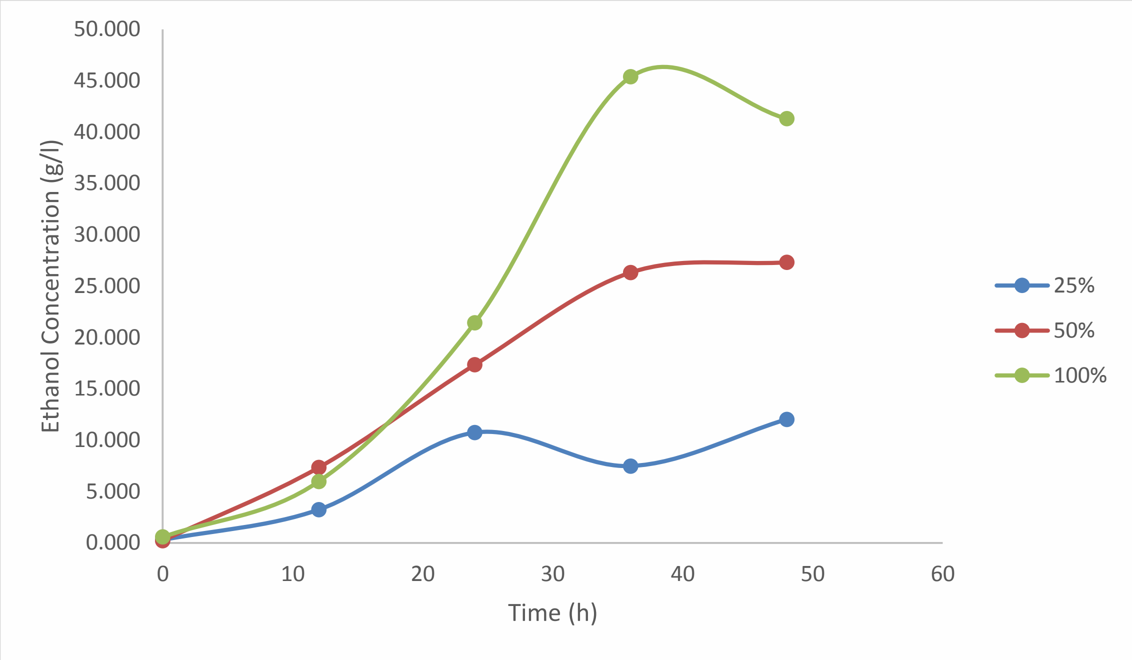

Figure 4.4: Bioethanol profile for the effect on OPF juice concentration

The highest bioethanol production is 45.36 g/l in 25% of OPF juice concentration at interval of 36 hour. This followed the OPF juice concentration of 50%, and 25% respectively with the bioethanol concentration of 27.31 g/l, and 12.02 g/l respectively.

The bioethanol depend to the OPF juice concentration according to Figure 4.4 shows the carbon source from the OPF juice is indeed the nutrient for yeast fermentation process and tend to consume abundant of carbon source to convert to bioethanol in absent of oxygen (Cheng *et al.*, 2002). This supported by Ranković *et al.* (2009) stated the increasing of fermentable sugars content from 5 to 13% (w/w), bioethanol yields increase for both applied substrates (thin juice and molasses). According to Imam and Capareda (2011), the maximum bioethanol production (% v/v) was 8.5 for V-1 and 9.5 for V-2 due to the different composition of carbon source for both V-1 and V-2.

4.6 Summary

The chapter concluded with an importance of standard curve of bioethanol for determination of bioethanol concentration for each effect of parameters study. The effect of each parameters study able to determine from the correlation between the peak area and the bioethanol concentration. The bioethanol production is supposed decreased according to the increasing value of pH for the effect of initial pH due to the different strain used from other research study and inaccuracy of pH adjustment. For the effect on agitation speed, the production of bioethanol is decreasing when the value of agitation speed increased because the presence of shear force in medium tend to break down the cells in medium. Then, the bioethanol production increased according to increase of OPF juice concentration due to the increasing of carbon source present in the OPF juice. Finally, the best parameter for the bioethanol production is in pH 7 for initial pH medium, agitation speed of 150 rpm, and 100% of OPF juice concentration.

5. CONCLUSION

5.1 Conclusion

The OPF contain the composition of sugar or carbon source similar as OPT sap. So, the OPF also be able to become raw material of the bioethanol fermentation. The OPF juice obtained from the pressing the OPF and proceed to the further research.

In this research, the parameters used are pH, agitation speed and OPF juice concentration. The bioethanol production is highest in the yeast natural or slightly acidic and decrease when more acidic of more alkali, the suitable agitation speed and the highest OPF juice concentration. Thus, the best condition of the bioethanol fermentation is in pH 7 of initial pH value, agitation speed of 150 rpm, and 100% v/v OPF juice concentration.

5.2 Recommendation

There are several points should recommend in order for further research in future. The method and procedure of experimental design should follows carefully. Any slacks on this part may affect to the result obtained and contradict with the theoretical in worst scenario.

The future result also should implied the microbial growth profile to show the longest timeline of the microbial chosen. This growth profile also able to determine the optimal timeline for the microbial growth to proceed with the bioethanol fermentation.

Besides that, the viscosity of the substrate also should be reconsider. The highest viscosity tend to slow down the agitation speed, especially implied in fermenter. Thus, the viscosity affect the best condition of bioethanol production in term of agitation speed.

Finally, this study have potential to scale up with the best condition require. Thus, this study proceed to 2 l fermenter with nitrogen purging inside since the bioethanol have good market demand in world.

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APPENDICES

GC-FID ANALYSATION

Initial pH Medium

pH 4

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    Acq. Instrument : Instrument 1                     Location : Vial 6
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                                                    Inj Volume : 1 µl
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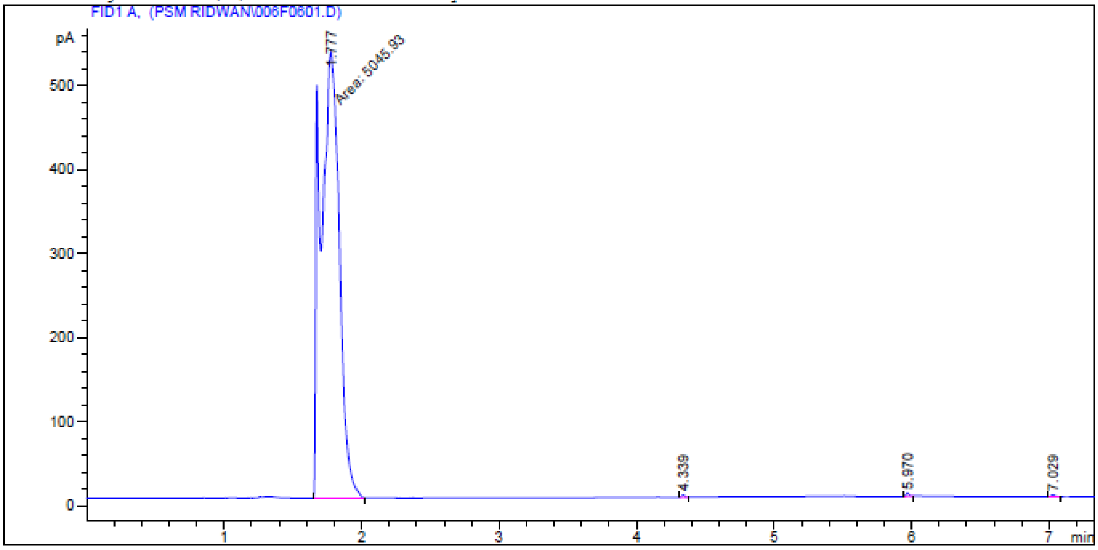


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    =====================================================================
                         Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A,

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.777 MM    0.1582 5045.92529  531.47144 99.74129
       2   4.339 BB    0.0205    3.78742    2.85119  0.07486
       3   5.970 BB    0.0244    6.30789    4.00904  0.12469
       4   7.029 BB    0.0268    2.99307    1.76342  0.05916

    Totals :                  5059.01367  540.09508

    =====================================================================
                         *** End of Report ***
```

Instrument 1 6/10/2013 4:16:25 PM hazimah   Page   1 of 1

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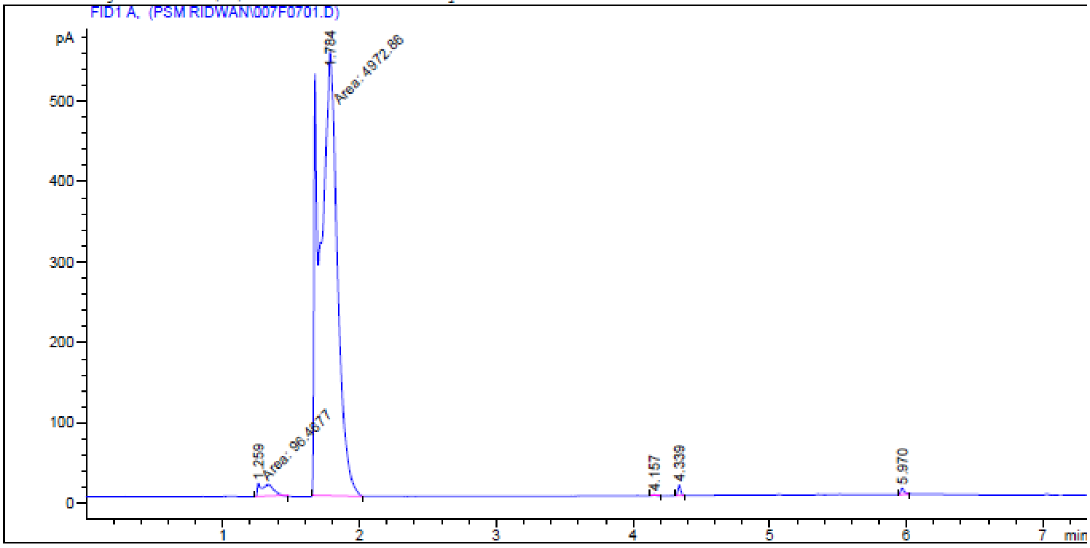


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    =====================================================================
                             Area Percent Report
    =====================================================================

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    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A,
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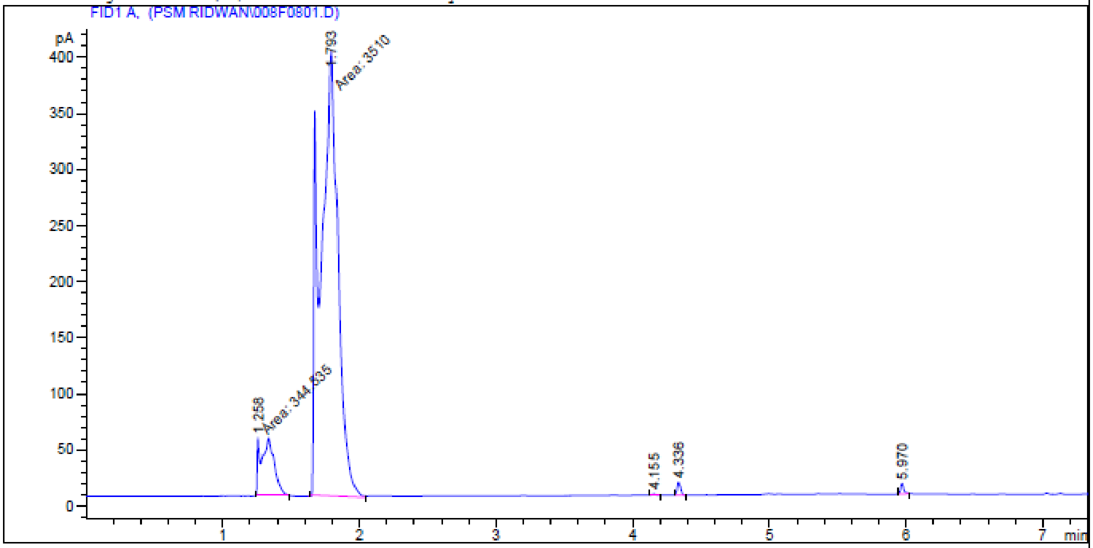
2	1.784	MM	0.1503	4972.85693	551.40533	97.44367
3	4.157	BB	0.0236	1.94579	1.29130	0.03813
4	4.339	BB	0.0218	18.29611	12.72533	0.35851
5	5.970	BB	0.0263	13.74780	7.89230	0.26939
Totals :				5103.31435	589.53230	

```
    =====================================================================
                             *** End of Report ***
```

Data File C:\CHEM32\1\DATA\PSM RIDWAN\008F0801.D
Sample Name: ph4 t2 d

===

```
Acq. Operator   : hazimah                        Seq. Line :   8
Acq. Instrument : Instrument 1                    Location : Vial 8
Injection Date  : 6/5/2013 2:31:24 PM                  Inj :   1
                                               Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```



===
Area Percent Report
===

```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: FID1 A,

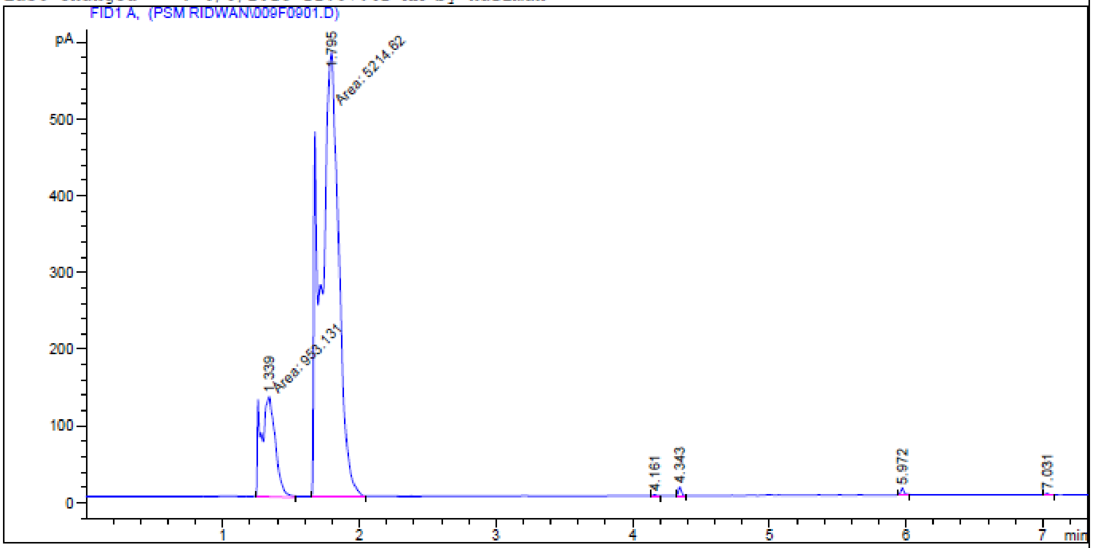
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.258	MM	0.1124	344.53540	51.07530	8.85123
2	1.793	MM	0.1477	3510.00000	396.07410	90.17304
3	4.155	BB	0.0243	1.95241	1.24343	0.05016
4	4.336	BB	0.0275	19.81752	11.82953	0.50912
5	5.970	BB	0.0258	16.21057	9.55812	0.41645

Totals : 3892.51590 469.78047

===
*** End of Report ***

Data File C:\CHEM32\1\DATA\PSM RIDWAN\009F0901.D
Sample Name: ph4 t3 d

```
=====================================================================
Acq. Operator   : hazimah                        Seq. Line :   9
Acq. Instrument : Instrument 1                    Location : Vial 9
Injection Date  : 6/5/2013 2:43:58 PM                  Inj :   1
                                               Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,
```

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.339	MM	0.1216	953.13147	130.66379	15.36181
2	1.795	MM	0.1502	5214.62207	578.52545	84.04513
3	4.161	BB	0.0235	1.85643	1.23528	0.02992
4	4.343	BB	0.0235	16.75757	11.15323	0.27009
5	5.972	BB	0.0267	14.77506	8.76745	0.23813
6	7.031	BB	0.0274	3.40729	1.94553	0.05492
Totals :				6204.54990	732.29073	

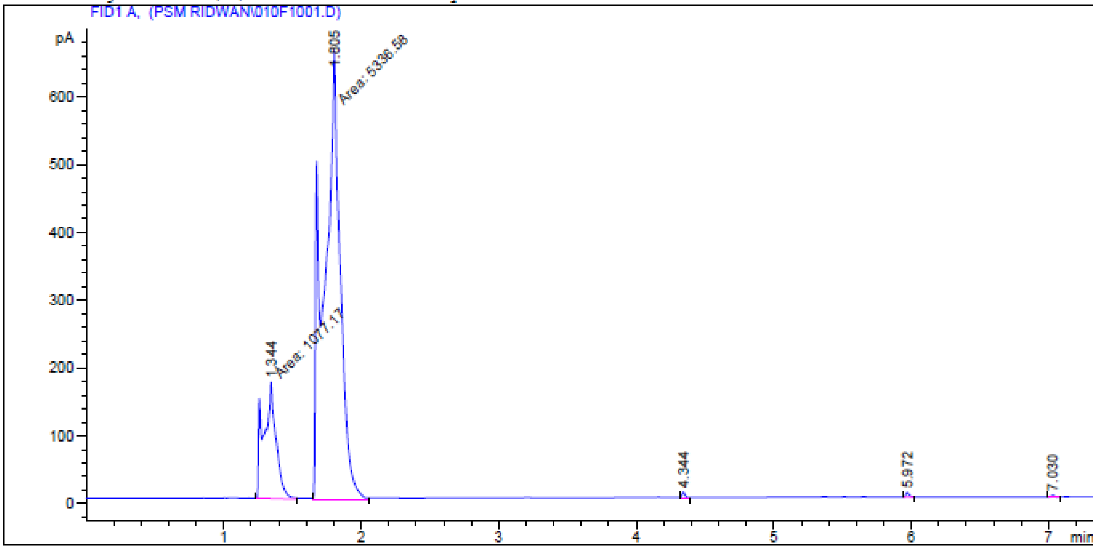
```
=====================================================================
                        *** End of Report ***
```

Instrument 1 6/10/2013 4:18:33 PM hazimah   Page   1 of 1

Data File C:\CHEM32\1\DATA\PSM RIDWAN\010F1001.D
Sample Name: ph4 t4 d

===

```
Acq. Operator   : hazimah                         Seq. Line :  10
Acq. Instrument : Instrument 1                     Location : Vial 10
Injection Date  : 6/5/2013 2:56:32 PM                   Inj :   1
                                                 Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```



===

Area Percent Report

===

```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.344	MM	0.1045	1077.16809	171.83539	16.72678
2	1.805	MM	0.1346	5336.57764	660.75110	82.86894
3	4.344	BB	0.0211	10.61351	7.68204	0.16481
4	5.972	BB	0.0259	10.95100	6.75187	0.17005
5	7.030	BB	0.0291	4.46993	2.47117	0.06941

Totals : 6439.78016 849.49157

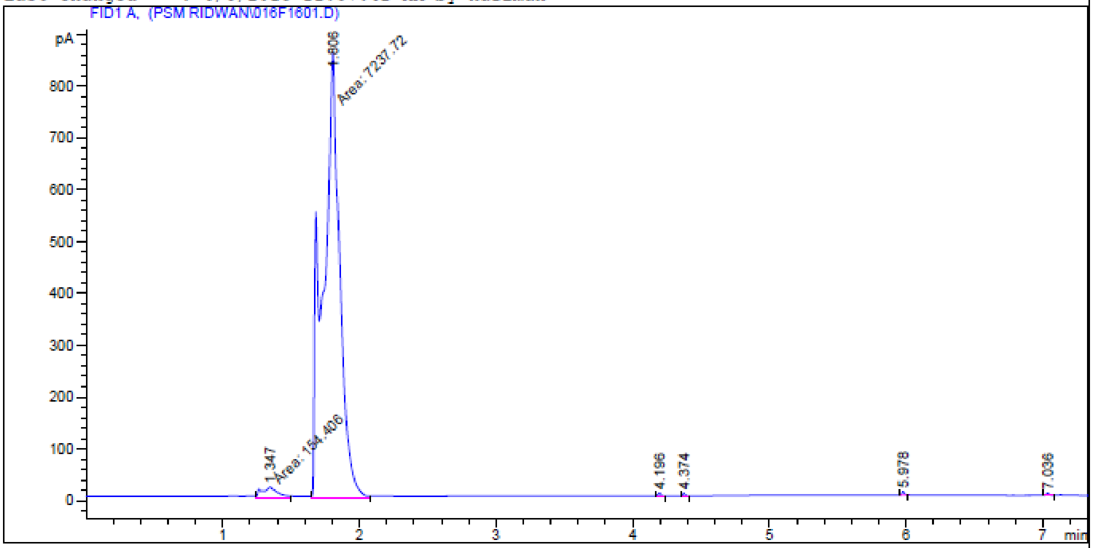
===

*** End of Report ***

pH 6

```
Data File C:\CHEM32\1\DATA\PSM RIDWAN\016F1601.D
 Sample Name: ph6 t0 d

    =====================================================================
    Acq. Operator   : hazimah                             Seq. Line :  16
    Acq. Instrument : Instrument 1                         Location : Vial 16
    Injection Date  : 6/5/2013 4:17:27 PM                       Inj :   1
                                                        Inj Volume : 1 µl
    Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
    Last changed    : 6/5/2013 1:01:42 PM by hazimah
    Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
    Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
    =====================================================================
                         Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A,

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.347 MM    0.1304  154.40614   19.73695  2.07997
       2   1.806 MM    0.1403 7237.71777  859.64813 97.49783
       3   4.196 BB    0.0219    8.09062    5.59524  0.10899
       4   4.374 BB    0.0190    6.23109    5.20635  0.08394
       5   5.978 BB    0.0211    9.68143    7.02890  0.13042
       6   7.036 BB    0.0261    7.33827    4.47845  0.09885

    Totals :                    7423.46531  901.69402

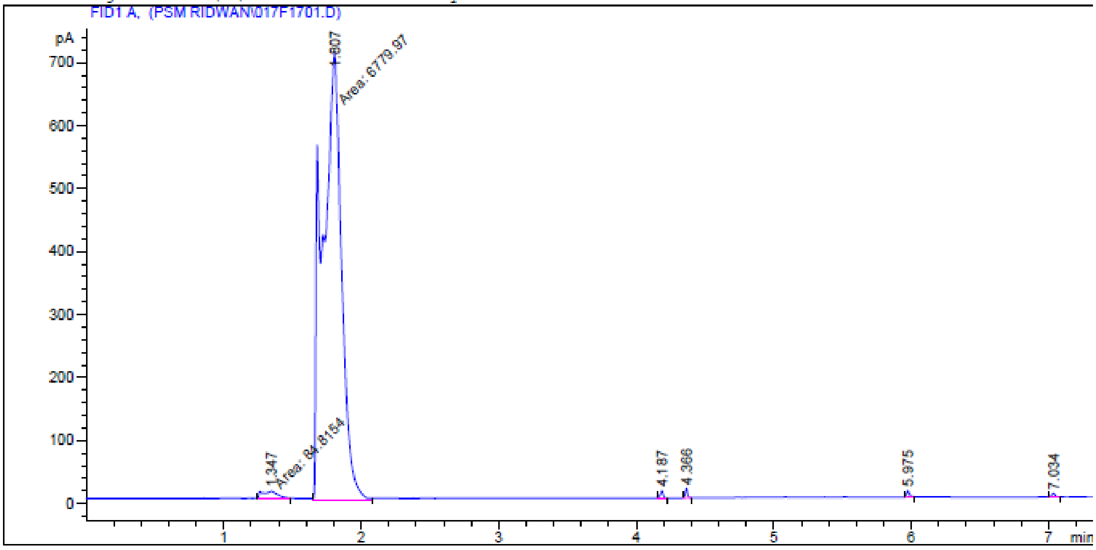
    =====================================================================
                             *** End of Report ***


Instrument 1 6/10/2013 4:29:29 PM hazimah                          Page    1 of 1
```

Data File C:\CHEM32\1\DATA\PSM RIDWAN\017F1701.D
Sample Name: ph6 t1 d

===

```
Acq. Operator   : hazimah                        Seq. Line :  17
Acq. Instrument : Instrument 1                    Location : Vial 17
Injection Date  : 6/5/2013 4:30:02 PM                  Inj :   1
                                                Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```



===
Area Percent Report
===

```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: FID1 A,

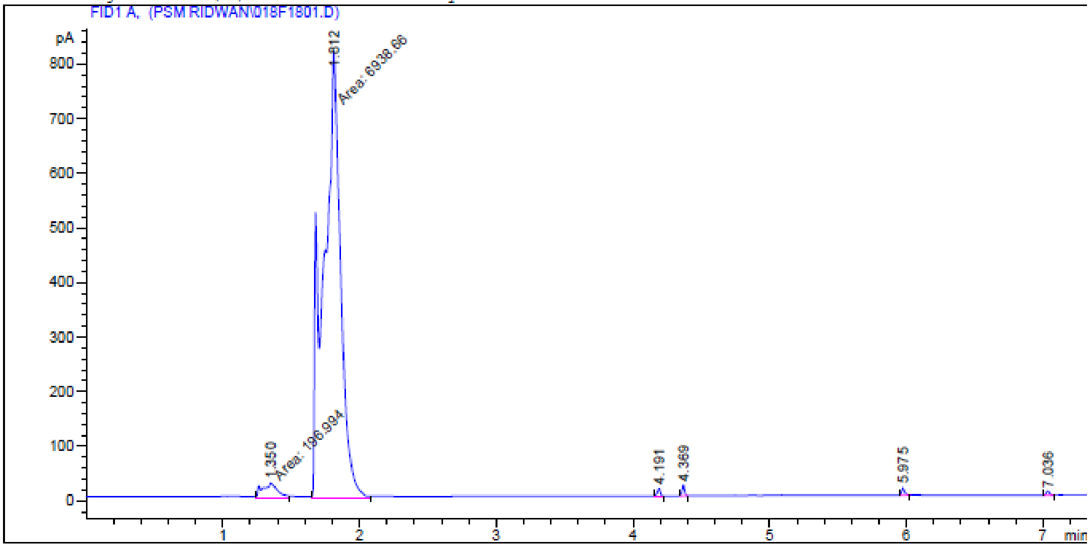
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.347	MM	0.1281	84.81538	11.03706	1.22534
2	1.807	MM	0.1590	6779.97314	710.77893	97.95100
3	4.187	BB	0.0227	15.56719	10.89353	0.22490
4	4.366	BB	0.0183	17.24758	15.07597	0.24918
5	5.975	BB	0.0219	14.45285	9.98193	0.20880
6	7.034	BB	0.0261	9.74450	5.64378	0.14078
Totals :				6921.80065	763.41119	

===
*** End of Report ***

Data File C:\CHEM32\1\DATA\PSM RIDWAN\018F1801.D
Sample Name: ph6 t2 d

===

Acq. Operator : hazimah Seq. Line : 18
Acq. Instrument : Instrument 1 Location : Vial 18
Injection Date : 6/5/2013 4:42:35 PM Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed : 6/5/2013 11:37:42 AM by hazimah



===

Area Percent Report

===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.350	MM	0.1236	196.99370	26.56111	2.73180
2	1.812	MM	0.1415	6938.66406	817.17017	96.22159
3	4.191	BB	0.0215	19.43445	13.78097	0.26951
4	4.369	BB	0.0186	22.93726	19.74741	0.31808
5	5.975	BB	0.0219	20.18608	13.96970	0.27993
6	7.036	BB	0.0263	12.91469	7.78948	0.17909

Totals : 7211.13023 899.01884

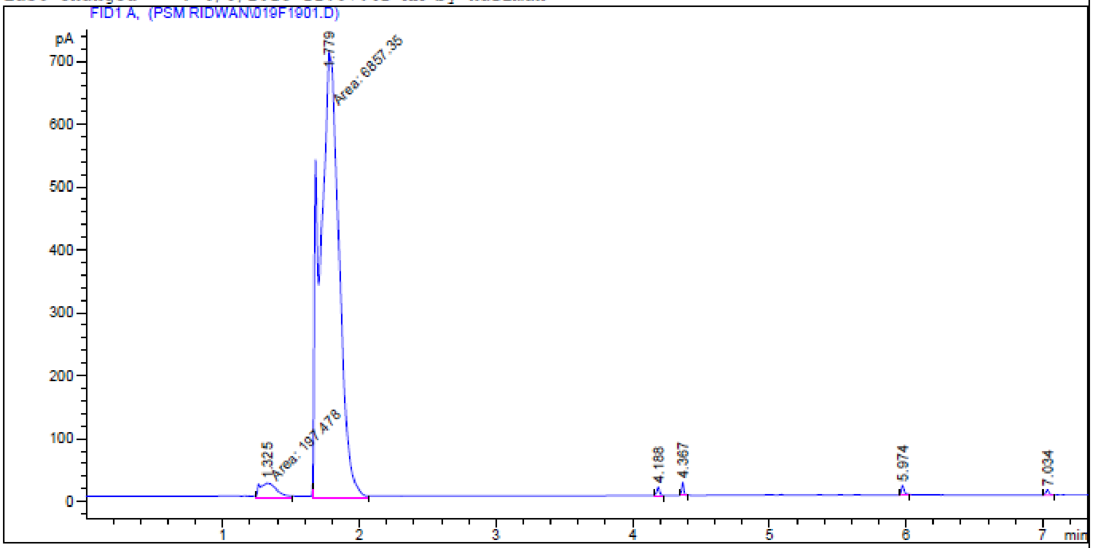
===

*** End of Report ***

Instrument 1 6/10/2013 4:30:51 PM hazimah Page 1 of 1

```
Data File C:\CHEM32\1\DATA\PSM RIDWAN\019F1901.D
 Sample Name: ph6 t3 d

    =====================================================================
    Acq. Operator   : hazimah                         Seq. Line :  19
    Acq. Instrument : Instrument 1                     Location : Vial 19
    Injection Date  : 6/5/2013 4:55:08 PM                   Inj :   1
                                                    Inj Volume : 1 µl
    Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
    Last changed    : 6/5/2013 1:01:42 PM by hazimah
    Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
    Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
    =====================================================================
                         Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A,

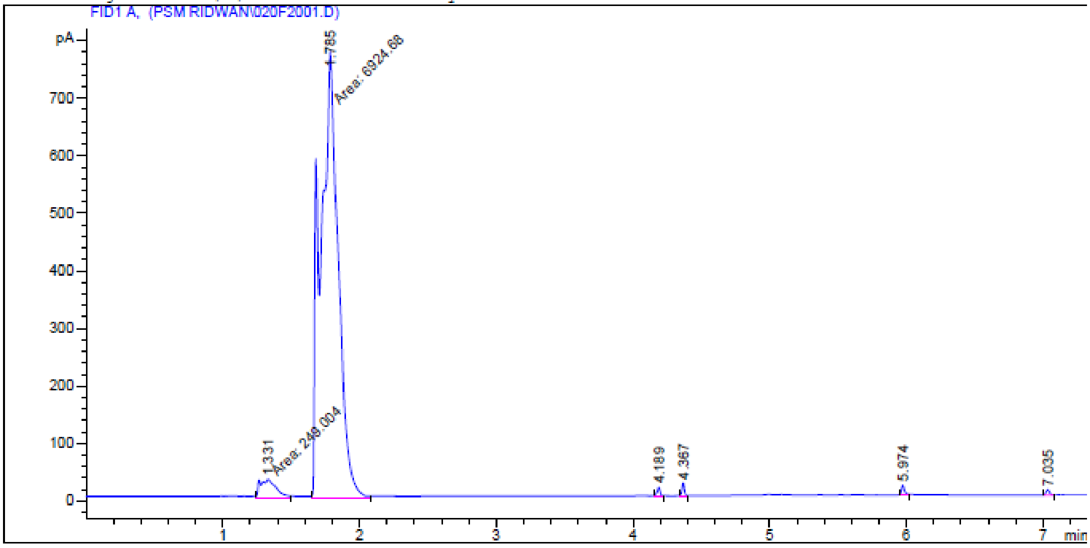
    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]    [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.325 MM    0.1436  197.47772   22.92178  2.76779
       2   1.779 MM    0.1614 6857.34814  708.03534 96.11058
       3   4.188 BB    0.0216   19.52087   13.72261  0.27360
       4   4.367 BB    0.0174   23.99304   20.92002  0.33628
       5   5.974 BB    0.0230   22.75564   14.72996  0.31894
       6   7.034 BB    0.0255   13.75742    8.23293  0.19282

    Totals :                    7134.85284  788.56264

    =====================================================================
                          *** End of Report ***
```

Data File C:\CHEM32\1\DATA\PSM RIDWAN\020F2001.D
Sample Name: ph6 t4 d

```
=====================================================================
Acq. Operator   : hazimah                        Seq. Line :  20
Acq. Instrument : Instrument 1                    Location : Vial 20
Injection Date  : 6/5/2013 5:07:42 PM                  Inj :   1
                                               Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,
```

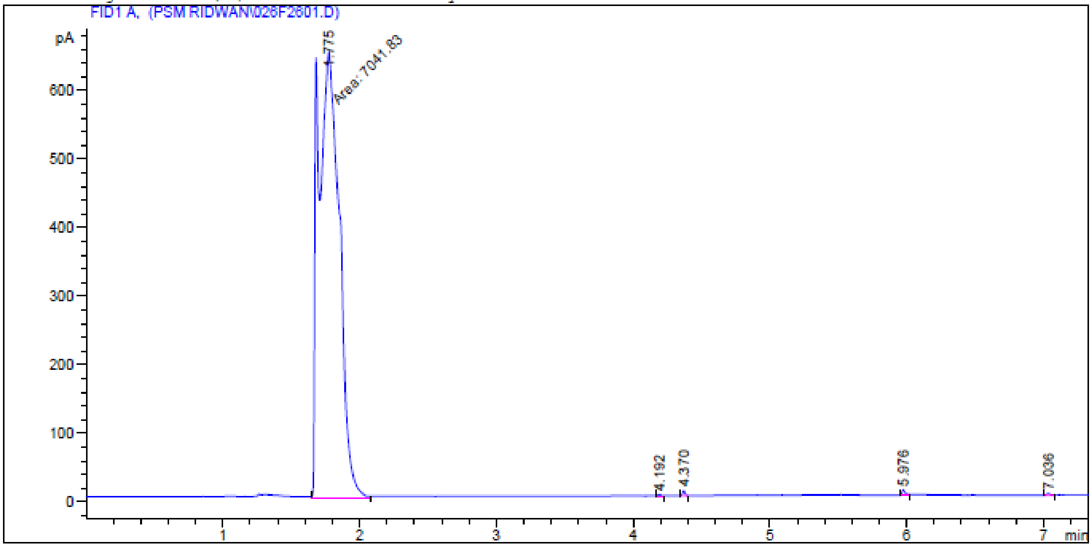
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.331	MM	0.1327	249.00359	31.28161	3.43016
2	1.785	MM	0.1489	6924.68213	775.22406	95.39123
3	4.189	BB	0.0217	20.31100	14.20391	0.27979
4	4.367	BB	0.0182	24.83073	21.86996	0.34206
5	5.974	BB	0.0222	24.61357	16.71777	0.33907
6	7.035	BB	0.0261	15.80304	9.63756	0.21770
Totals :				7259.24405	868.93487	

```
=====================================================================
                          *** End of Report ***
```

pH 7

```
Data File C:\CHEM32\1\DATA\PSM RIDWAN\026F2601.D
 Sample Name: ph7 t0 d

    =====================================================================
    Acq. Operator   : hazimah                             Seq. Line :  26
    Acq. Instrument : Instrument 1                         Location : Vial 26
    Injection Date  : 6/5/2013 6:23:11 PM                       Inj :   1
                                                         Inj Volume : 1 µl
    Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
    Last changed    : 6/5/2013 1:01:42 PM by hazimah
    Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
    Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A,

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]       [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.775 MM    0.1804 7041.82617  650.39789 99.62831
       2   4.192 BB    0.0196    2.42552    1.81828  0.03432
       3   4.370 BB    0.0171    8.36023    7.42432  0.11828
       4   5.976 BB    0.0218   11.40439    7.94106  0.16135
       5   7.036 BB    0.0271    4.08144    2.37266  0.05774

    Totals :                  7068.09776  669.95420

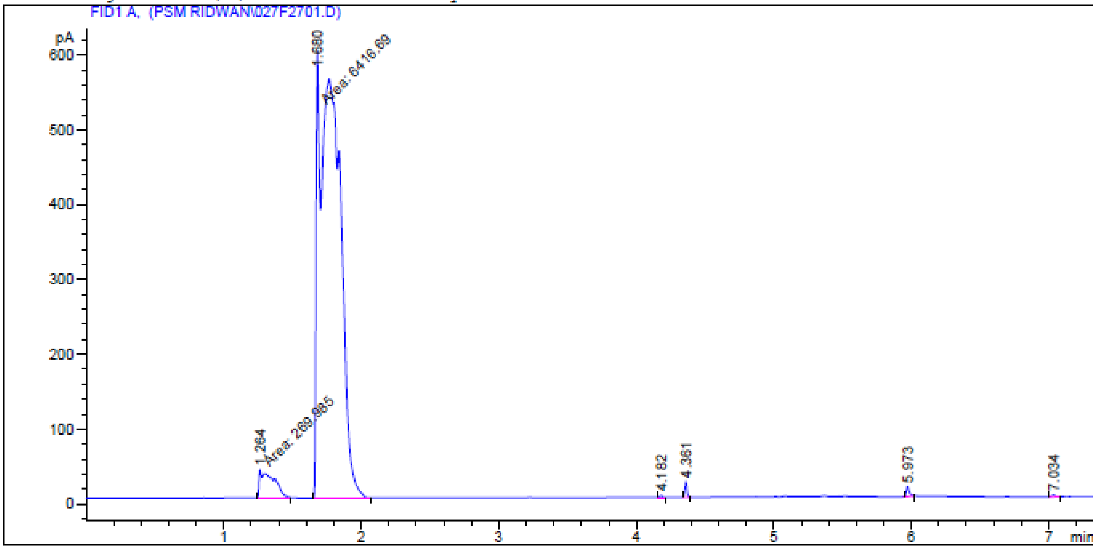

    =====================================================================
                              *** End of Report ***



Instrument 1 6/10/2013 4:37:42 PM hazimah                          Page    1 of 1
```

Data File C:\CHEM32\1\DATA\PSM RIDWAN\027F2701.D
Sample Name: ph7 t1 d

```
=====================================================================
Acq. Operator   : hazimah                        Seq. Line :  27
Acq. Instrument : Instrument 1                    Location : Vial 27
Injection Date  : 6/5/2013 6:35:40 PM                  Inj :   1
                                               Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,
```

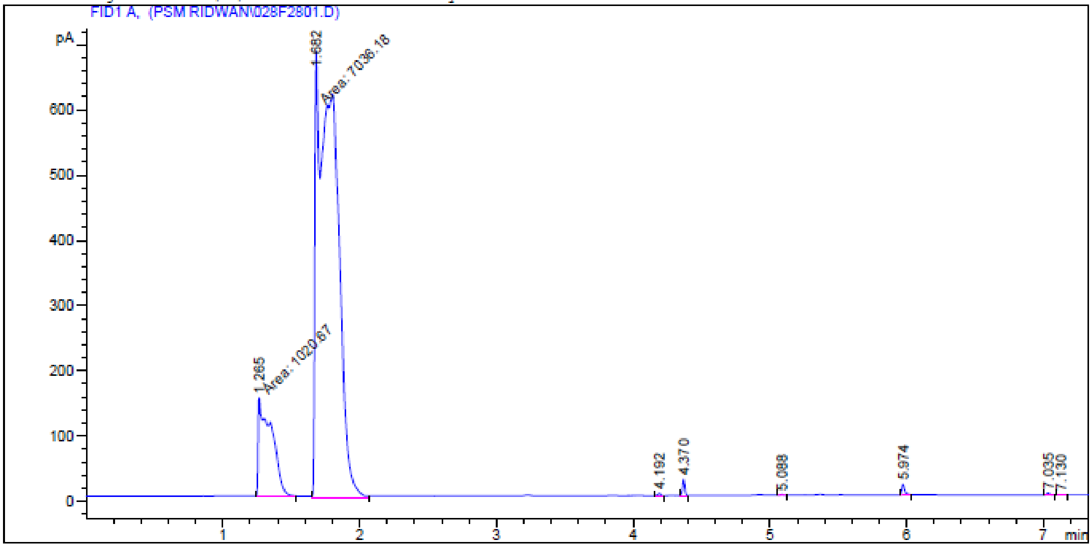
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.264	MM	0.1192	269.98538	37.76423	4.00722
2	1.680	MM	0.1791	6416.68799	597.11987	95.23884
3	4.182	BB	0.0197	3.19033	2.37481	0.04735
4	4.361	BB	0.0180	23.49296	21.01754	0.34869
5	5.973	BB	0.0241	20.44164	13.18299	0.30340
6	7.034	BB	0.0273	3.67169	2.10577	0.05450
Totals :				6737.46998	673.56521	

```
=====================================================================
                          *** End of Report ***
```

Data File C:\CHEM32\1\DATA\PSM RIDWAN\028F2801.D
Sample Name: ph7 t2 d

===

```
Acq. Operator   : hazimah                          Seq. Line :  28
Acq. Instrument : Instrument 1                      Location : Vial 28
Injection Date  : 6/5/2013 6:48:14 PM                     Inj :   1
                                                  Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```



===
Area Percent Report
===

```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: FID1 A,

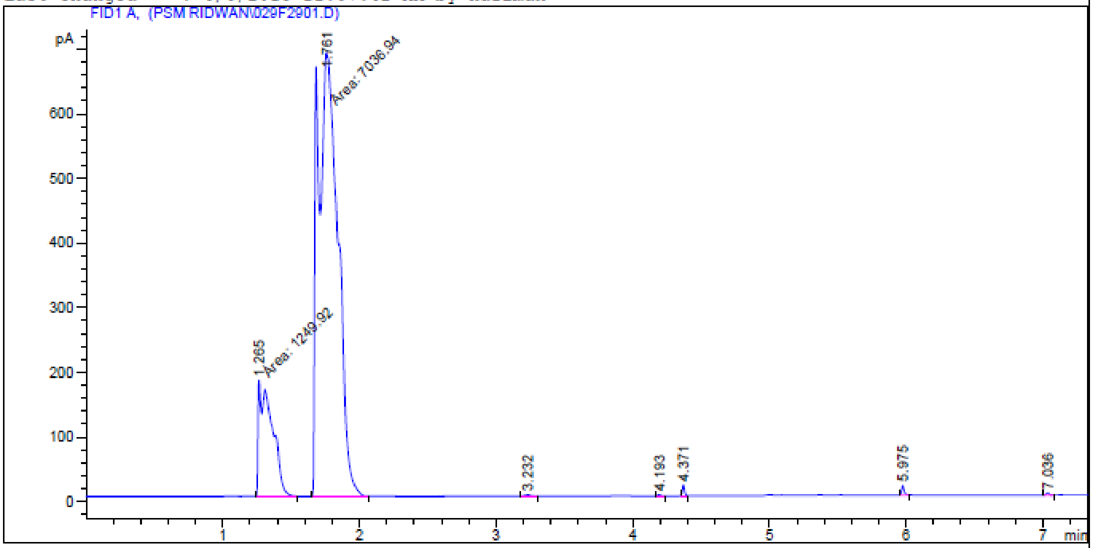
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.265	MM	0.1128	1020.66821	150.82683	12.56557
2	1.682	MM	0.1715	7036.18018	683.79919	86.62327
3	4.192	BB	0.0214	4.81769	3.42577	0.05931
4	4.370	BB	0.0170	27.40515	24.61930	0.33739
5	5.088	BB	0.0183	1.29909	1.05642	0.01599
6	5.974	BB	0.0229	24.50525	16.01323	0.30169
7	7.035	BB	0.0272	5.58063	3.22850	0.06870
8	7.130	BB	0.0273	2.28094	1.25000	0.02808
Totals :				8122.73713	884.21925	

===
*** End of Report ***

Instrument 1 6/10/2013 4:39:09 PM hazimah   Page 1 of 1

Data File C:\CHEM32\1\DATA\PSM RIDWAN\029F2901.D
Sample Name: ph7 t3 d

```
=====================================================================
Acq. Operator   : hazimah                        Seq. Line :  29
Acq. Instrument : Instrument 1                    Location : Vial 29
Injection Date  : 6/5/2013 7:00:47 PM                  Inj :   1
                                               Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,
```

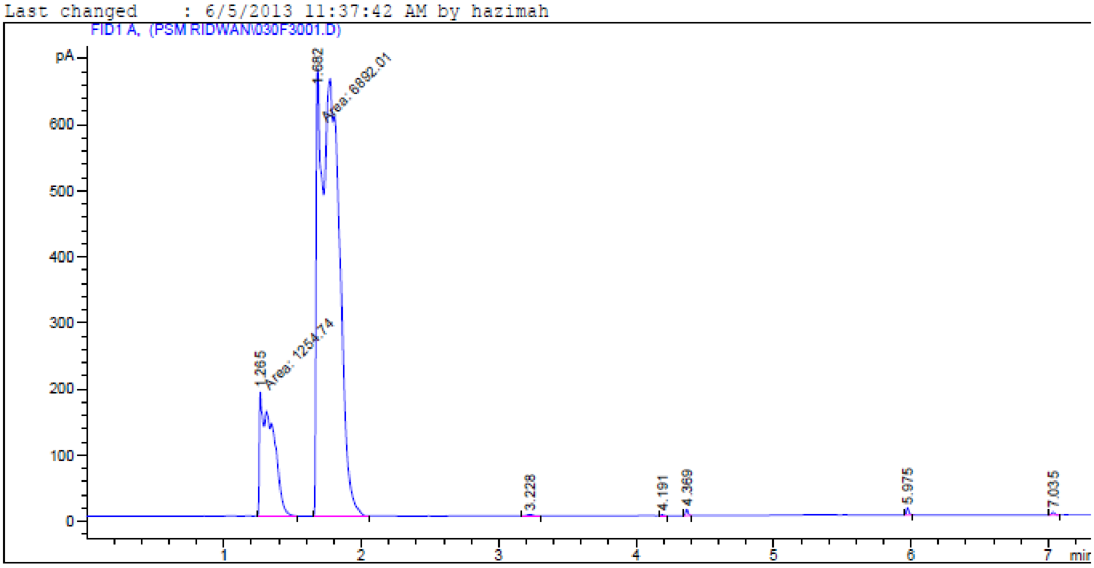
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.265	MM	0.1157	1249.92017	180.07866	14.98665
2	1.761	MM	0.1707	7036.94434	687.23303	84.37354
3	3.232	BB	0.0457	5.73104	1.92533	0.06872
4	4.193	BB	0.0208	2.31434	1.71138	0.02775
5	4.371	BB	0.0181	18.91279	16.87454	0.22677
6	5.975	BB	0.0208	19.77852	14.60251	0.23715
7	7.036	BB	0.0250	6.62481	4.06718	0.07943
Totals :				8340.22601	906.49264	

```
=====================================================================
                         *** End of Report ***
```

Instrument 1 6/10/2013 4:40:08 PM hazimah   Page   1 of 1

Data File C:\CHEM32\1\DATA\PSM RIDWAN\030F3001.D
Sample Name: ph7 t4 d

===
Acq. Operator : hazimah Seq. Line : 30
Acq. Instrument : Instrument 1 Location : Vial 30
Injection Date : 6/5/2013 7:13:20 PM Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed : 6/5/2013 11:37:42 AM by hazimah



===
Area Percent Report
===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.265	MM	0.1115	1254.74048	187.50566	15.32489
2	1.682	MM	0.1698	6892.00684	676.58258	84.17615
3	3.228	BB	0.0473	6.08989	2.01224	0.07438
4	4.191	BB	0.0207	1.88996	1.40401	0.02308
5	4.369	BB	0.0184	10.89349	9.46365	0.13305
6	5.975	BB	0.0205	14.51438	10.91263	0.17727
7	7.035	BB	0.0265	7.46531	4.47664	0.09118

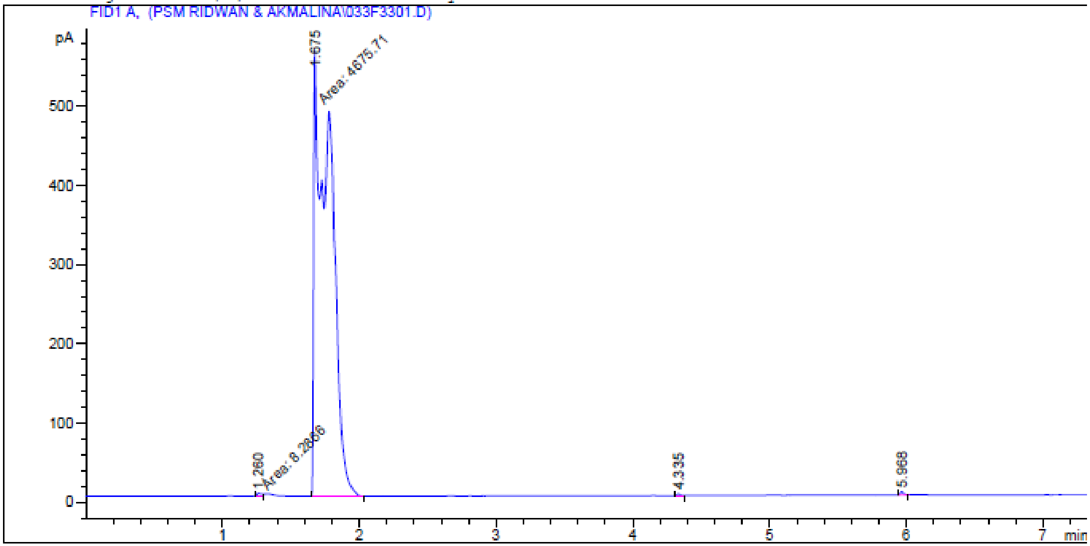
Totals : 8187.60034 892.35741

===
*** End of Report ***

pH 8

```
Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\033F3301.D
 Sample Name: ph8 t0 d

    =====================================================================
    Acq. Operator   : hazimah                        Seq. Line :  33
    Acq. Instrument : Instrument 1                    Location : Vial 33
    Injection Date  : 6/6/2013 4:19:08 AM                  Inj :   1
                                                    Inj Volume : 1 µl
    Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
    Last changed    : 6/5/2013 1:01:42 PM by hazimah
    Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
    Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A,

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]    [pA*s]      [pA]       %
    ----|-------|----|-------|----------|----------|--------|
       1   1.260 MM    0.0352    8.28660    3.92576  0.17650
       2   1.675 MM    0.1385 4675.70996  562.76520 99.58733
       3   4.335 BB    0.0217    3.62774    2.53508  0.07727
       4   5.968 BB    0.0254    7.46086    4.49580  0.15891

    Totals :                   4695.08516  573.72183


    =====================================================================
                              *** End of Report ***

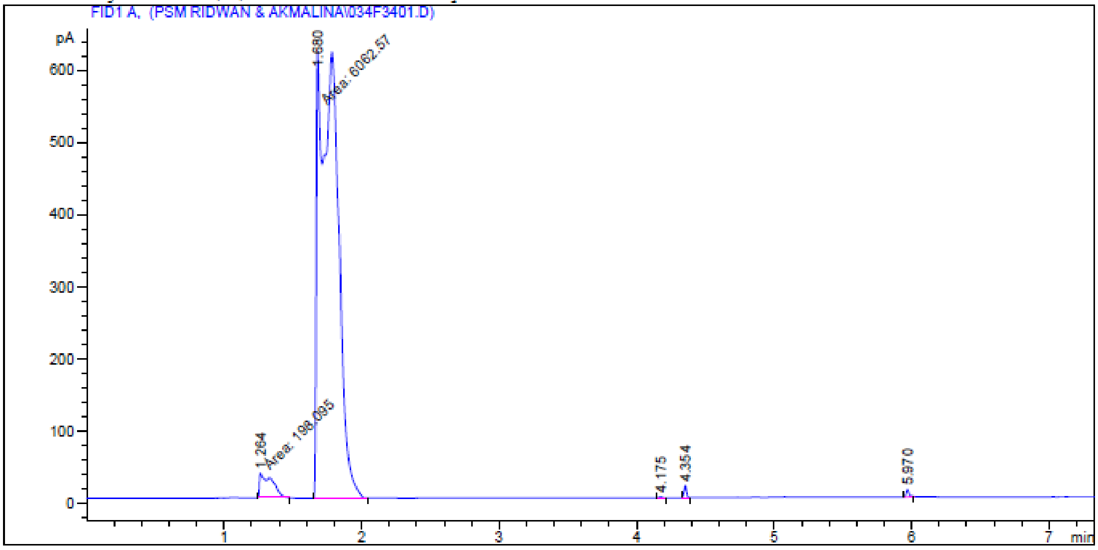


Instrument 1 6/10/2013 4:46:46 PM hazimah                            Page    1 of 1
```

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\034F3401.D
Sample Name: ph8 t1 d

===

Acq. Operator : hazimah Seq. Line : 34
Acq. Instrument : Instrument 1 Location : Vial 34
Injection Date : 6/6/2013 4:31:42 AM Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed : 6/5/2013 11:37:42 AM by hazimah



===

Area Percent Report

===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.264	MM	0.1003	198.09512	32.92737	3.14617
2	1.680	MM	0.1633	6062.57373	618.88385	96.28643
3	4.175	BB	0.0212	1.69006	1.21402	0.02684
4	4.354	BB	0.0186	18.60487	15.94516	0.29548
5	5.970	BB	0.0236	15.43129	10.24443	0.24508

Totals : 6296.39507 679.21483

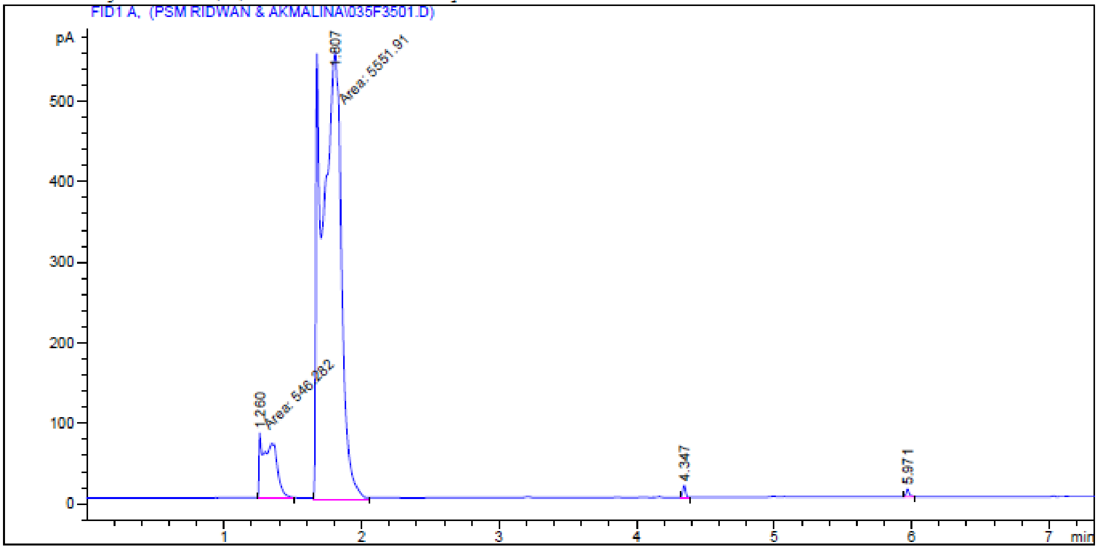
===

*** End of Report ***

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\035F3501.D
Sample Name: ph8 t2 d

===

```
Acq. Operator   : hazimah                        Seq. Line :  35
Acq. Instrument : Instrument 1                    Location : Vial 35
Injection Date  : 6/6/2013 4:44:14 AM                  Inj :   1
                                                Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```



===
Area Percent Report
===

```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

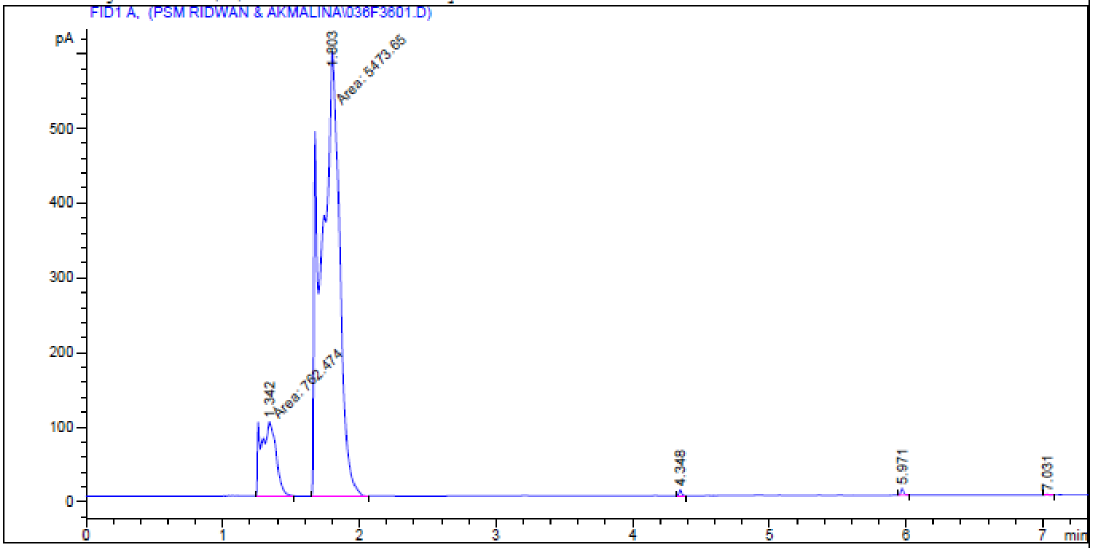
Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.260	MM	0.1130	546.28247	80.54099	8.90776
2	1.807	MM	0.1666	5551.91406	555.52466	90.53028
3	4.347	BB	0.0197	19.00473	15.08023	0.30989
4	5.971	BB	0.0253	15.45844	9.36552	0.25207
Totals :				6132.65971	660.51140	

===
*** End of Report ***

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\036F3601.D
Sample Name: ph8 t3 d

```
=====================================================================
Acq. Operator   : hazimah                        Seq. Line :  36
Acq. Instrument : Instrument 1                    Location : Vial 36
Injection Date  : 6/6/2013 4:56:51 AM                  Inj :   1
                                               Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

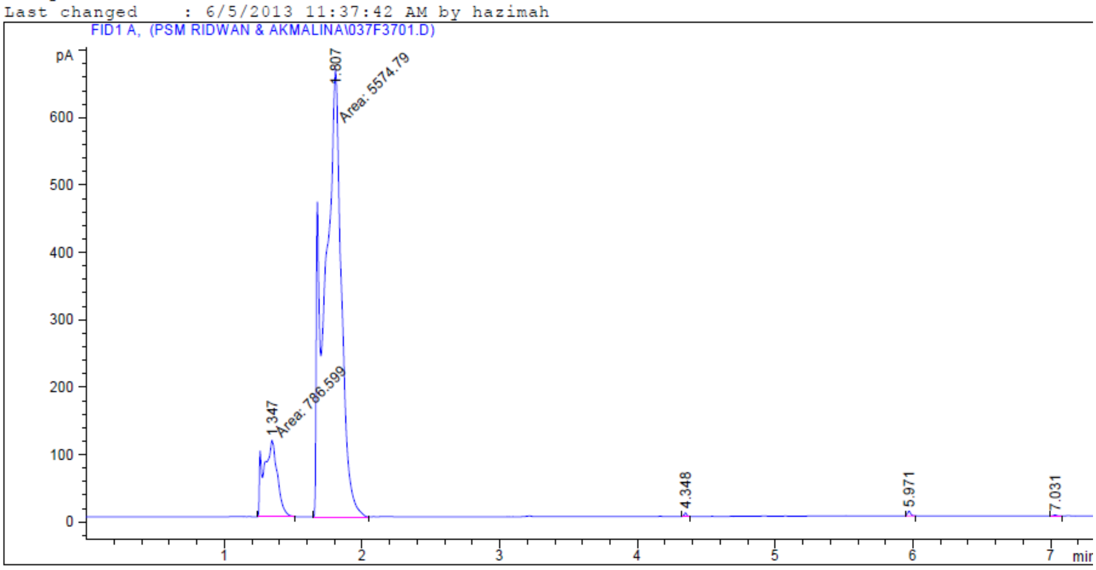
Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.342	MM	0.1275	762.47382	99.66074	12.17527
2	1.803	MM	0.1534	5473.65186	594.85944	87.40393
3	4.348	BB	0.0197	10.31496	8.16793	0.16471
4	5.971	BB	0.0250	13.70951	8.90747	0.21892
5	7.031	BB	0.0272	2.32753	1.34375	0.03717
Totals :				6262.47767	712.93932	

```
=====================================================================
                         *** End of Report ***
```

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\037F3701.D
Sample Name: ph8 t4 d

===
Acq. Operator : hazimah Seq. Line : 37
Acq. Instrument : Instrument 1 Location : Vial 37
Injection Date : 6/6/2013 5:09:28 AM Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed : 6/5/2013 11:37:42 AM by hazimah



===
Area Percent Report
===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.347	MM	0.1155	786.59851	113.52827	12.32589
2	1.807	MM	0.1395	5574.79248	666.19781	87.35624
3	4.348	BB	0.0196	6.67109	5.31486	0.10454
4	5.971	BB	0.0240	10.95563	7.10234	0.17167
5	7.031	BB	0.0276	2.65850	1.50473	0.04166

Totals : 6381.67620 793.64802

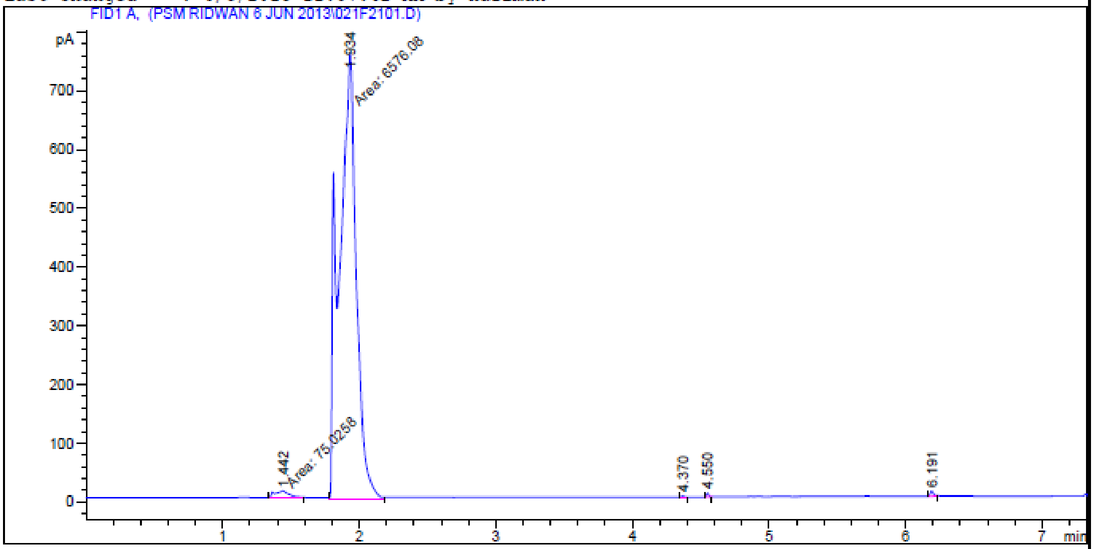
===
*** End of Report ***

Agitation Speed

150 rpm

```
Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\021F2101.D
 Sample Name: 150rpm (2) t0

    =====================================================================
    Acq. Operator   : hazimah                         Seq. Line :  21
    Acq. Instrument : Instrument 1                     Location : Vial 21
    Injection Date  : 6/6/2013 9:56:12 PM                   Inj :   1
                                                     Inj Volume : 1 µl
    Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
    Last changed    : 6/6/2013 5:43:30 PM by hazimah
    Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
    Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A,

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.442 MM    0.1108   75.02584   11.28731  1.12417
       2   1.934 MM    0.1445 6576.08252  758.64093 98.53444
       3   4.370 BB    0.0210    2.14403    1.55914  0.03213
       4   4.550 BB    0.0173    7.61402    6.69611  0.11409
       5   6.191 BB    0.0231   13.02628    8.87067  0.19518

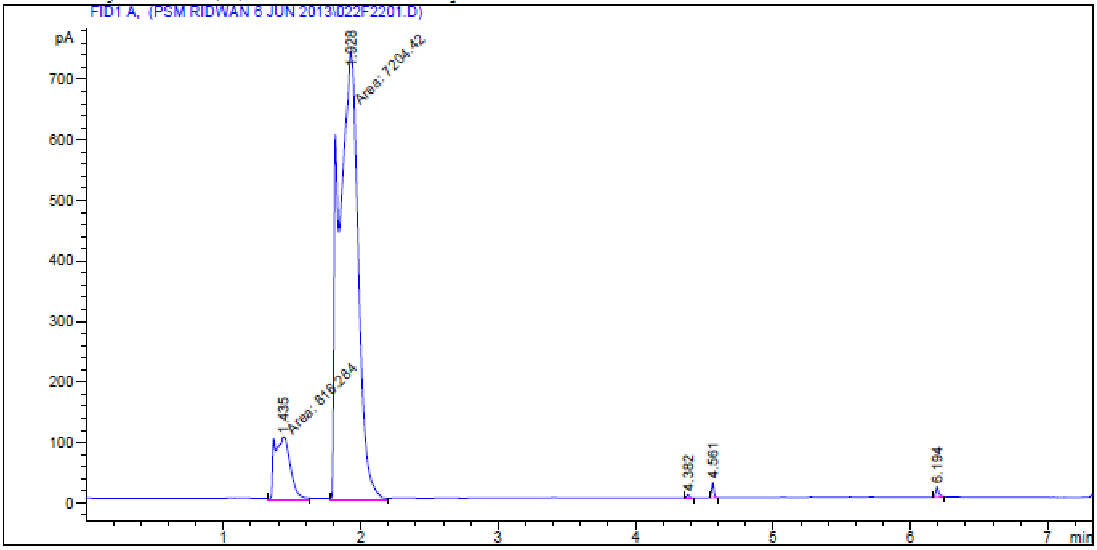
    Totals :                  6673.89270  787.05415

    =====================================================================
                              *** End of Report ***


Instrument 1 6/10/2013 5:41:41 PM hazimah                            Page    1 of 1
```

Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\022F2201.D
Sample Name: 150rpm (2) t1

===
Acq. Operator   : hazimah   Seq. Line : 22
Acq. Instrument : Instrument 1   Location : Vial 22
Injection Date : 6/6/2013 10:08:45 PM   Inj :   1
                                                Inj Volume : 1 µl
Acq. Method   : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed   : 6/6/2013 5:43:30 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed   : 6/5/2013 11:37:42 AM by hazimah



===
Area Percent Report
===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.435	MM	0.1320	816.28394	103.08543	10.10155
2	1.928	MM	0.1623	7204.41846	739.95404	89.15500
3	4.382	BB	0.0219	7.33088	5.05162	0.09072
4	4.561	BB	0.0181	28.05550	25.02450	0.34719
5	6.194	BB	0.0234	24.69054	16.51331	0.30555

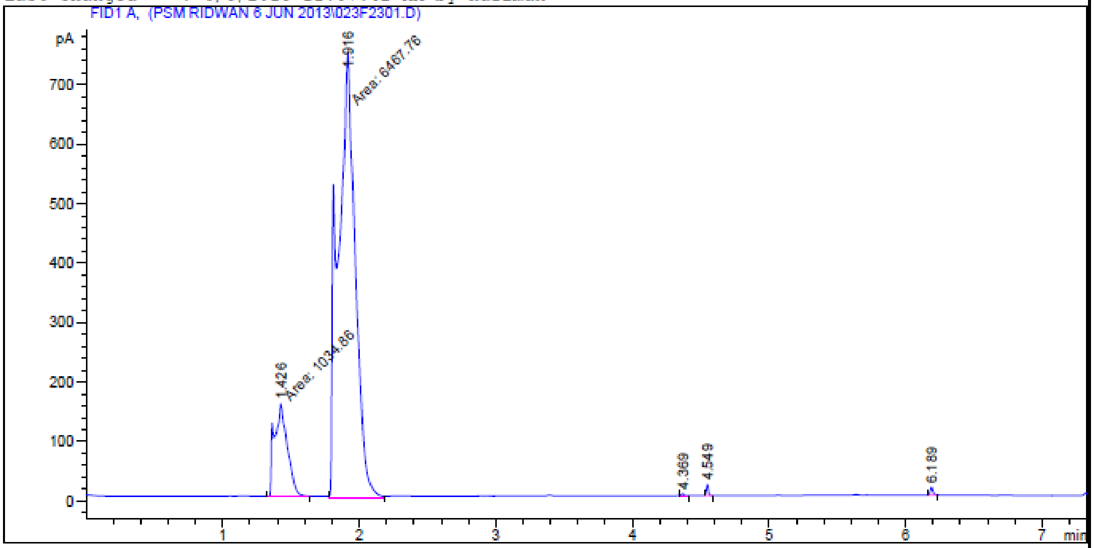
Totals : 8080.77932 889.62890

===
*** End of Report ***

Instrument 1 6/10/2013 5:42:31 PM hazimah   Page 1 of 1

Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\023F2301.D
Sample Name: 150rpm (2) t2

```
=====================================================================
Acq. Operator   : hazimah                        Seq. Line :  23
Acq. Instrument : Instrument 1                    Location : Vial 23
Injection Date  : 6/6/2013 10:21:14 PM                  Inj :   1
                                               Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/6/2013 5:43:30 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,
```

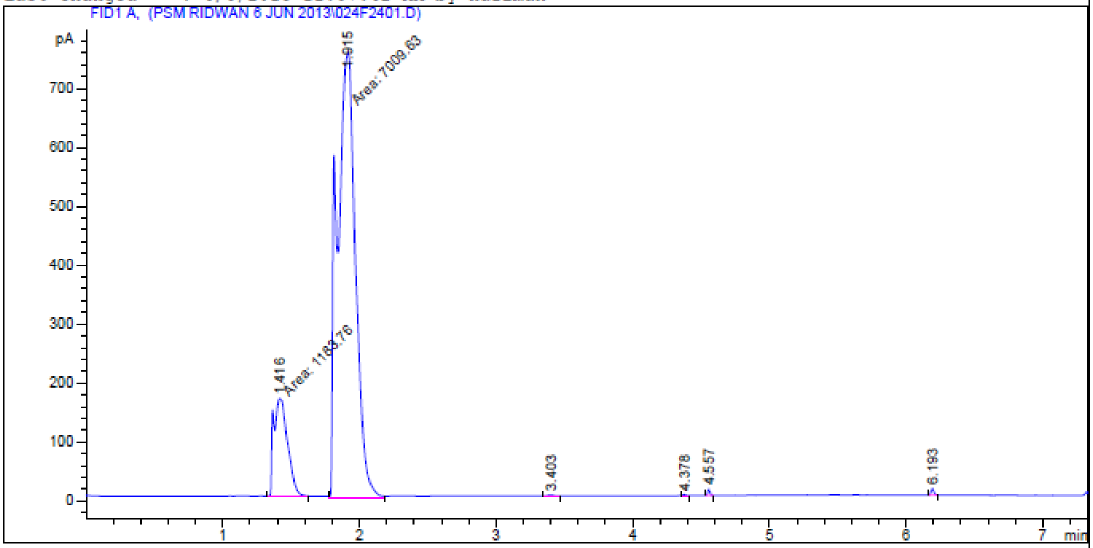
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.426	MM	0.1104	1034.86279	156.19902	13.71176
2	1.916	MM	0.1440	6467.76172	748.35284	85.69679
3	4.369	BB	0.0209	5.06655	3.71970	0.06713
4	4.549	BB	0.0178	20.70983	18.86631	0.27440
5	6.189	BB	0.0217	18.86146	13.17262	0.24991
Totals :				7547.26236	940.31049	

```
=====================================================================
                         *** End of Report ***
```

Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\024F2401.D
Sample Name: 150rpm (2) t3

===

```
Acq. Operator   : hazimah                        Seq. Line :  24
Acq. Instrument : Instrument 1                    Location : Vial 24
Injection Date  : 6/6/2013 10:33:43 PM                 Inj :   1
                                                Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/6/2013 5:43:30 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```



===
Area Percent Report
===

```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: FID1 A,

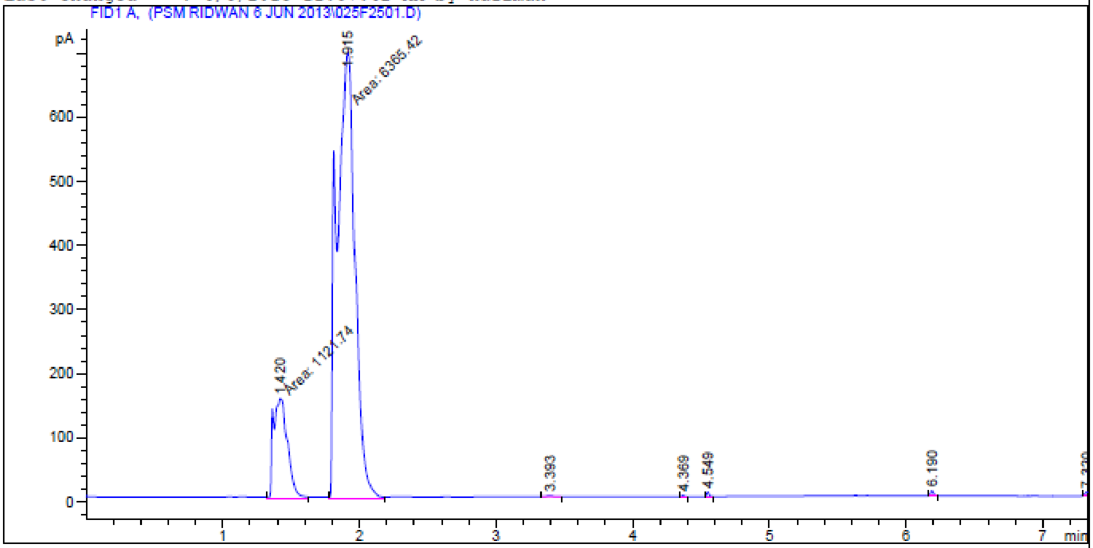
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.416	MM	0.1190	1183.75635	165.72414	14.38429
2	1.915	MM	0.1547	7009.62549	755.07111	85.17673
3	3.403	BB	0.0392	4.72989	1.71413	0.05747
4	4.378	BB	0.0207	2.77357	2.06015	0.03370
5	4.557	BB	0.0186	13.14409	11.30435	0.15972
6	6.193	BB	0.0213	15.47814	11.05414	0.18808

Totals : 8229.50752 946.92801

===
*** End of Report ***

```
Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\025F2501.D
 Sample Name: 150rpm (2) t4

    =====================================================================
    Acq. Operator   : hazimah                          Seq. Line :  25
    Acq. Instrument : Instrument 1                      Location : Vial 25
    Injection Date  : 6/6/2013 10:46:15 PM                   Inj :   1
                                                      Inj Volume : 1 µl
    Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
    Last changed    : 6/6/2013 5:43:30 PM by hazimah
    Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
    Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A,

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.420 MM    0.1206 1121.73535  154.96980 14.91306
       2   1.915 MM    0.1526 6365.41504  695.00922 84.62588
       3   3.393 BB    0.0433    4.60134    1.71420  0.06117
       4   4.369 BB    0.0211    2.16674    1.56856  0.02881
       5   4.549 BB    0.0183    7.82550    6.86471  0.10404
       6   6.190 BB    0.0227   11.61495    8.10395  0.15442
       7   7.320 BBA   0.0237    8.47140    5.96102  0.11262

    Totals :                  7521.83031  874.19146

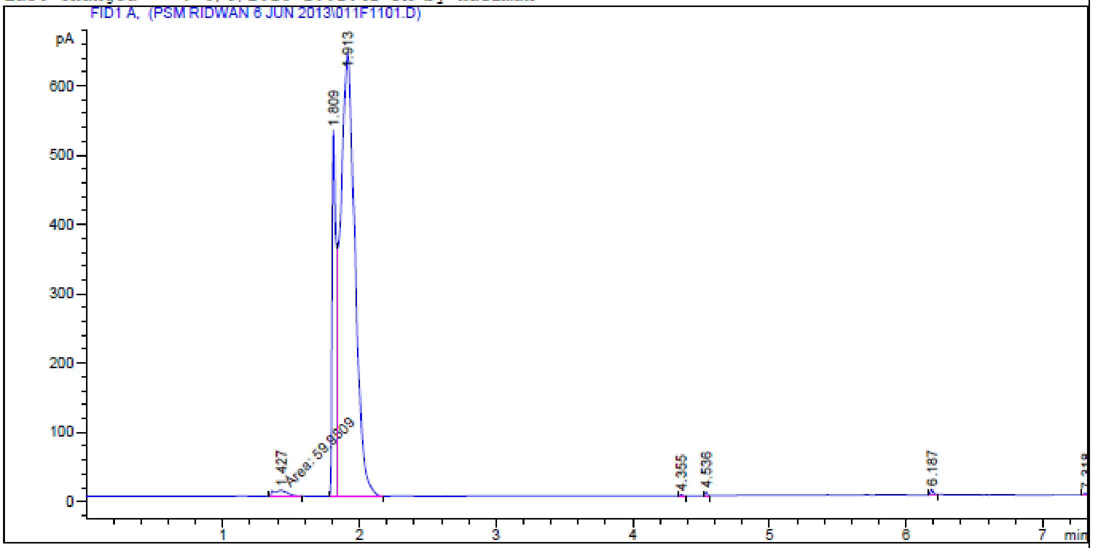

    =====================================================================
                             *** End of Report ***


Instrument 1 6/10/2013 5:44:50 PM hazimah                          Page    1 of 1
```

180 rpm

```
Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\011F1101.D
 Sample Name: 180rpm (2) t0

    =====================================================================
    Acq. Operator   : hazimah                         Seq. Line :  11
    Acq. Instrument : Instrument 1                     Location : Vial 11
    Injection Date  : 6/6/2013 7:50:23 PM                   Inj :   1
                                                     Inj Volume : 1 µl
    Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
    Last changed    : 6/6/2013 5:43:30 PM by hazimah
    Analysis Method : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
    Last changed    : 6/5/2013 1:01:42 PM by hazimah
```




```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A,

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]    [pA*s]     [pA]         %
    ----|-------|----|-------|----------|----------|--------|
       1   1.427 MM     0.1179   59.98088    8.47936  1.03466
       2   1.809 BV     0.0287 1071.82581  528.73401 18.48879
       3   1.913 VB     0.0965 4639.72119  640.31769 80.03429
       4   4.355 BB     0.0201    2.30203    1.77278  0.03971
       5   4.536 BB     0.0170    6.32486    5.68645  0.10910
       6   6.187 BB     0.0219   11.44988    7.89779  0.19751
       7   7.318 BBA    0.0237    5.56223    3.65982  0.09595

    Totals :                   5797.16688 1196.54789

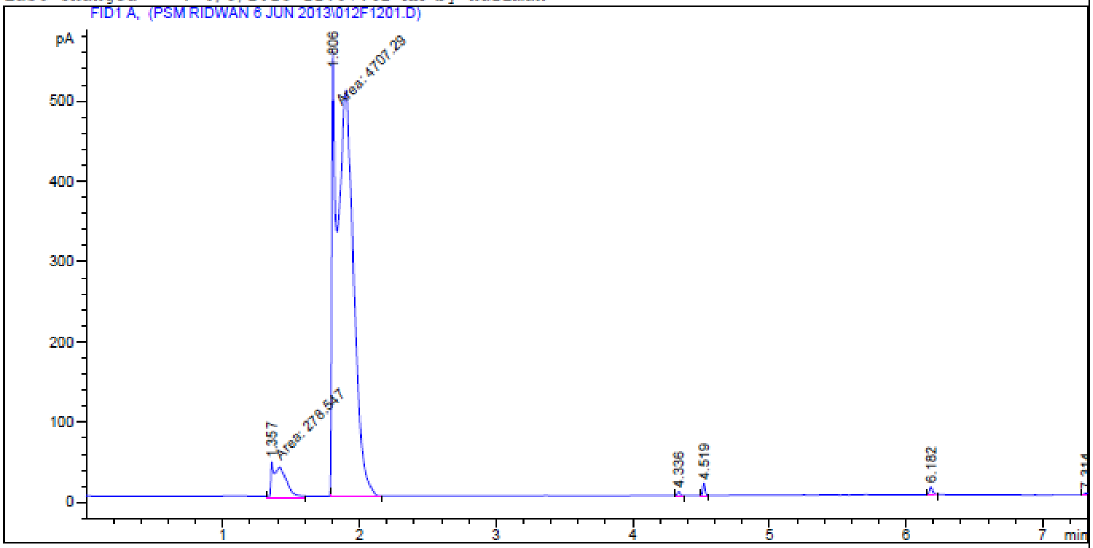

    =====================================================================
                              *** End of Report ***


Instrument 1 6/7/2013 11:58:41 AM hazimah                                   Page    1 of 1
```

Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\012F1201.D
Sample Name: 180rpm (2) t1

===

```
Acq. Operator   : hazimah                        Seq. Line :  12
Acq. Instrument : Instrument 1                    Location : Vial 12
Injection Date  : 6/6/2013 8:02:56 PM                  Inj :   1
                                               Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/6/2013 5:43:30 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```



===
Area Percent Report
===

```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: FID1 A,

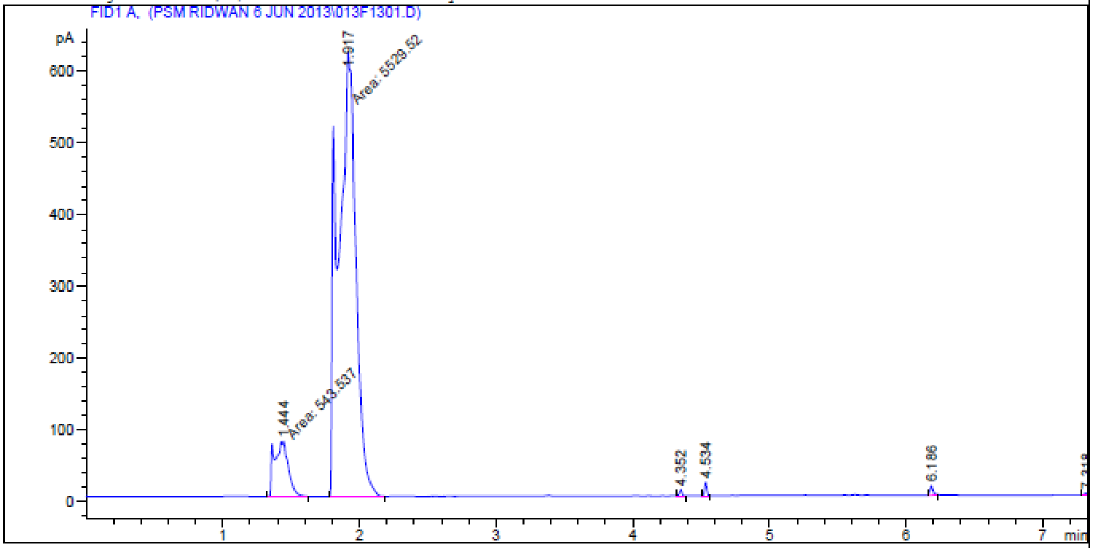
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.357	MM	0.1057	278.54666	43.90378	5.53778
2	1.806	MM	0.1416	4707.29297	553.91931	93.58558
3	4.336	BB	0.0216	6.94554	5.18062	0.13808
4	4.519	BB	0.0188	17.89023	15.12848	0.35568
5	6.182	BB	0.0249	15.17258	9.38199	0.30165
6	7.314	BBA	0.0262	4.08633	2.47927	0.08124
Totals :				5029.93432	629.99345	

===
*** End of Report ***

Instrument 1 6/10/2013 5:30:59 PM hazimah   Page   1 of 1

Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\013F1301.D
Sample Name: 180rpm (2) t2

===
Acq. Operator : hazimah   Seq. Line : 13
Acq. Instrument : Instrument 1   Location : Vial 13
Injection Date : 6/6/2013 8:15:28 PM   Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed : 6/6/2013 5:43:30 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed : 6/5/2013 11:37:42 AM by hazimah



===
Area Percent Report
===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

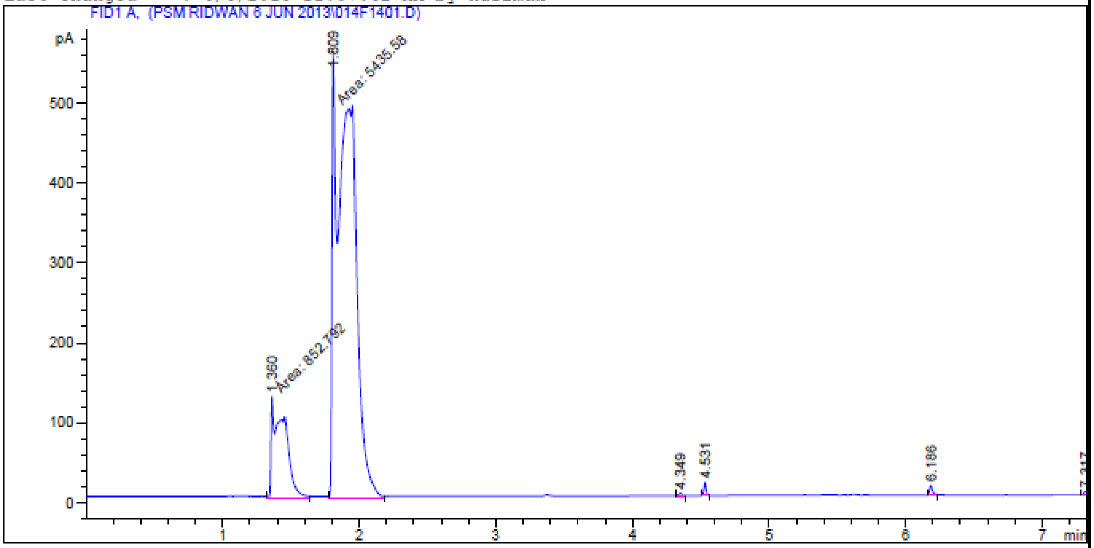
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.444	MM	0.1187	543.53693	76.29793	8.86611
2	1.917	MM	0.1487	5529.51660	619.90814	90.19686
3	4.352	BB	0.0204	11.02182	8.35643	0.17979
4	4.534	BB	0.0184	21.34299	18.62331	0.34814
5	6.186	BB	0.0240	19.58651	12.71687	0.31949
6	7.318	BBA	0.0257	5.49303	3.55900	0.08960
Totals :				6130.49788	739.46168	

===
*** End of Report ***

Instrument 1 6/10/2013 5:31:46 PM hazimah   Page 1 of 1

```
Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\014F1401.D
 Sample Name: 180rpm (2) t3

    =====================================================================
    Acq. Operator   : hazimah                        Seq. Line :  14
    Acq. Instrument : Instrument 1                    Location : Vial 14
    Injection Date  : 6/6/2013 8:28:03 PM                  Inj :   1
                                                    Inj Volume : 1 µl
    Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
    Last changed    : 6/6/2013 5:43:30 PM by hazimah
    Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
    Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A,

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.360 MM    0.1119  852.79230  127.00481 13.45866
       2   1.809 MM    0.1625 5435.58496  557.65405 85.78374
       3   4.349 BB    0.0211    4.51499    3.26883  0.07125
       4   4.531 BB    0.0166   17.65555   16.39229  0.27864
       5   6.186 BB    0.0238   18.00253   11.81899  0.28411
       6   7.317 BBA   0.0260    7.83088    5.02254  0.12359

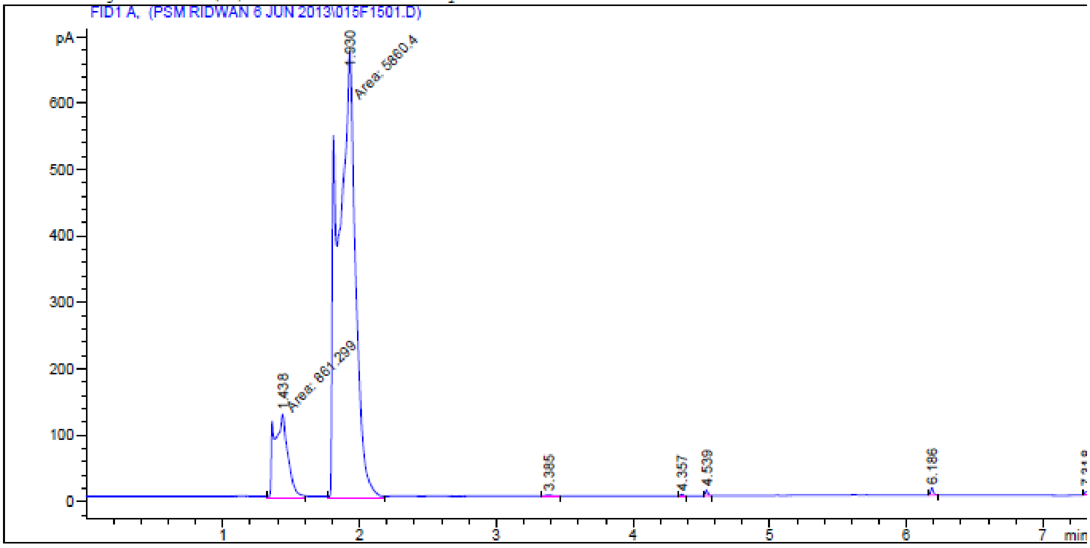
    Totals :                  6336.38121  721.16150


    =====================================================================
                              *** End of Report ***


Instrument 1 6/10/2013 5:32:30 PM hazimah                          Page    1 of 1
```

Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\015F1501.D
Sample Name: 180rpm (2) t4

```
=====================================================================
Acq. Operator   : hazimah                         Seq. Line :  15
Acq. Instrument : Instrument 1                     Location : Vial 15
Injection Date  : 6/6/2013 8:40:39 PM                   Inj :   1
                                                 Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/6/2013 5:43:30 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,
```

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.438	MM	0.1145	861.29877	125.34070	12.73097
2	1.930	MM	0.1455	5860.40039	671.11743	86.62332
3	3.385	BB	0.0450	4.33406	1.53027	0.06406
4	4.357	BB	0.0204	2.65445	2.01542	0.03924
5	4.539	BB	0.0184	10.54098	9.14436	0.15581
6	6.186	BB	0.0228	15.94684	11.05601	0.23571
7	7.318	BBA	0.0249	10.20841	6.82806	0.15089
Totals :				6765.38391	827.03225	

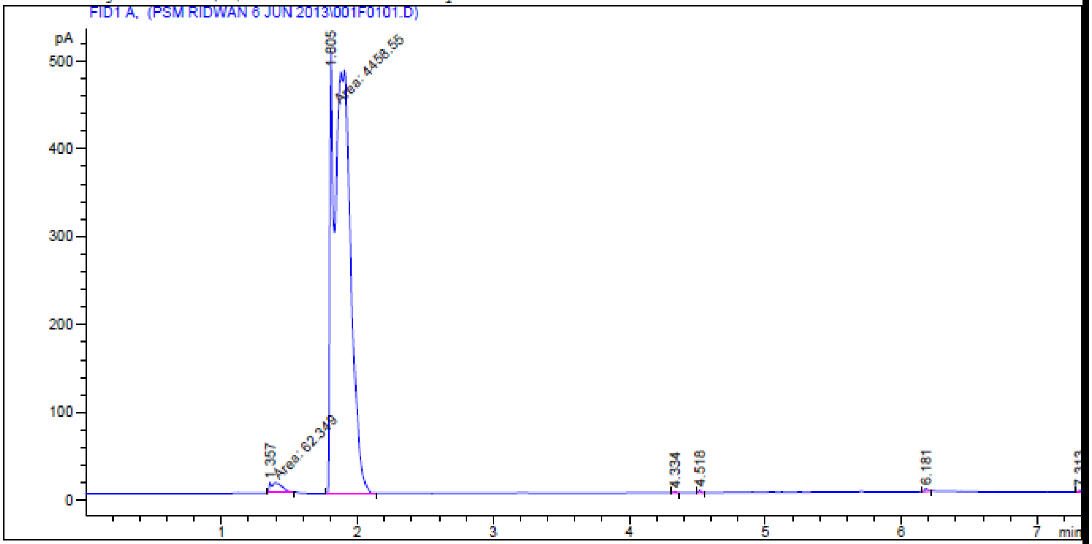
```
=====================================================================
                         *** End of Report ***
```

Instrument 1 6/10/2013 5:34:19 PM hazimah   Page   1 of 1

210 rpm

```
Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\001F0101.D
 Sample Name: 210rpm (2) t0

    =====================================================================
    Acq. Operator   : hazimah                          Seq. Line :   1
    Acq. Instrument : Instrument 1                      Location : Vial 1
    Injection Date  : 6/6/2013 5:44:54 PM                    Inj :   1
                                                      Inj Volume : 1 µl
    Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
    Last changed    : 6/6/2013 5:43:30 PM by hazimah
    Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
    Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs

    Signal 1: FID1 A,
```

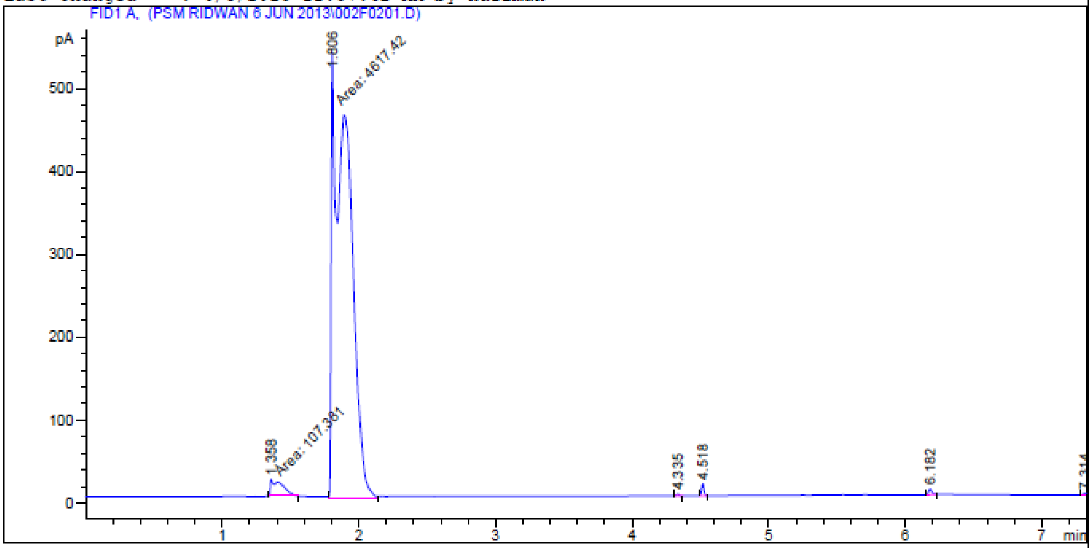
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.357	MM	0.0952	62.34902	10.91233	1.37549
2	1.805	MM	0.1474	4458.54980	504.09912	98.36101
3	4.334	BB	0.0205	1.85237	1.39446	0.04087
4	4.518	BB	0.0184	2.97455	2.59273	0.06562
5	6.181	BB	0.0246	4.81389	3.02454	0.10620
6	7.313	BBA	0.0262	2.30289	1.40257	0.05080
Totals :				4532.84252	523.42575	

```
    =====================================================================
                             *** End of Report ***
```

Instrument 1 6/10/2013 5:14:46 PM hazimah   Page   1 of 1

Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\002F0201.D
Sample Name: 210rpm (2) t1

```
=====================================================================
Acq. Operator   : hazimah                         Seq. Line :   2
Acq. Instrument : Instrument 1                     Location : Vial 2
Injection Date  : 6/6/2013 5:57:24 PM                   Inj :   1
                                                Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/6/2013 5:43:30 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
=====================================================================
                       Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,
```

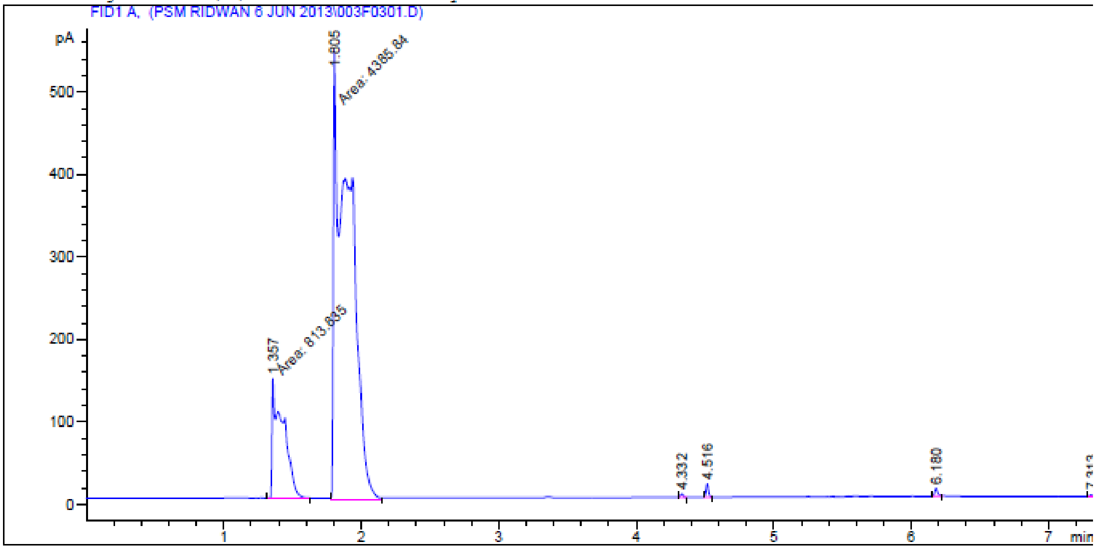
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.358	MM	0.0920	107.38122	19.44274	2.25648
2	1.806	MM	0.1432	4617.42090	537.38525	97.02930
3	4.335	BB	0.0201	2.90209	2.24043	0.06098
4	4.518	BB	0.0186	16.96576	14.50642	0.35651
5	6.182	BB	0.0251	11.20122	6.85828	0.23538
6	7.314	BBA	0.0258	2.91905	1.81332	0.06134
Totals :				4758.79023	582.24645	

```
=====================================================================
                         *** End of Report ***
```

Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\003F0301.D
Sample Name: 210rpm (2) t2

===

```
Acq. Operator   : hazimah                        Seq. Line :   3
Acq. Instrument : Instrument 1                    Location : Vial 3
Injection Date  : 6/6/2013 6:10:01 PM                  Inj :   1
                                                Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/6/2013 5:43:30 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```



===

Area Percent Report

===

```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.357	MM	0.0937	813.83453	144.72620	15.51840
2	1.805	MM	0.1345	4385.84375	543.66693	83.63034
3	4.332	BB	0.0204	5.44070	4.11124	0.10374
4	4.516	BB	0.0193	19.89981	16.24734	0.37945
5	6.180	BB	0.0242	14.51665	9.28986	0.27681
6	7.313	BBA	0.0275	4.78573	2.86412	0.09126

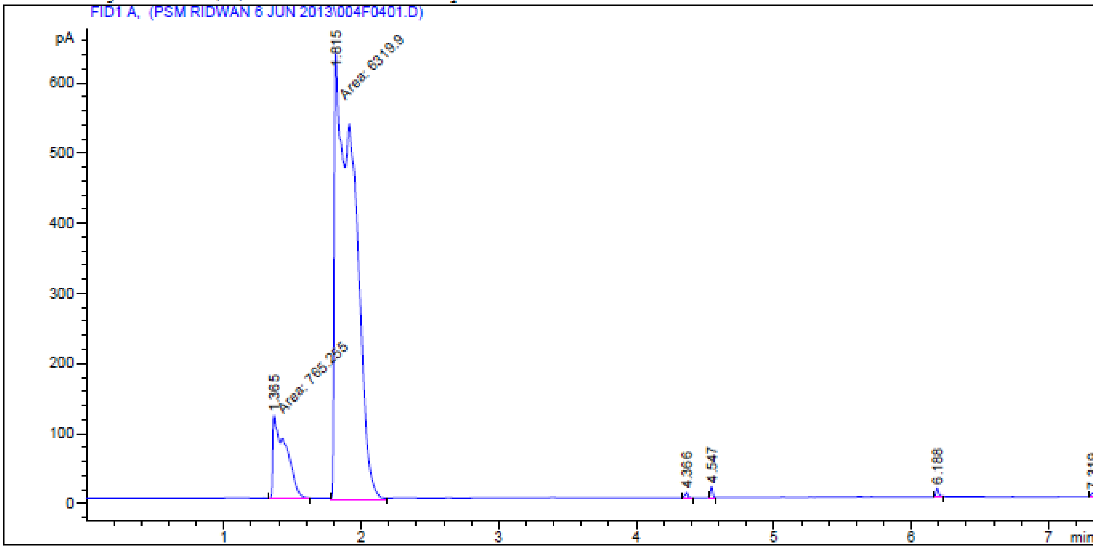
Totals : 5244.32116 720.90568

===

*** End of Report ***

```
Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\004F0401.D
 Sample Name: 210rpm (2) t3

    =====================================================================
    Acq. Operator   : hazimah                        Seq. Line :   4
    Acq. Instrument : Instrument 1                    Location : Vial 4
    Injection Date  : 6/6/2013 6:22:34 PM                  Inj :   1
                                                    Inj Volume : 1 µl
    Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
    Last changed    : 6/6/2013 5:43:30 PM by hazimah
    Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
    Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A,

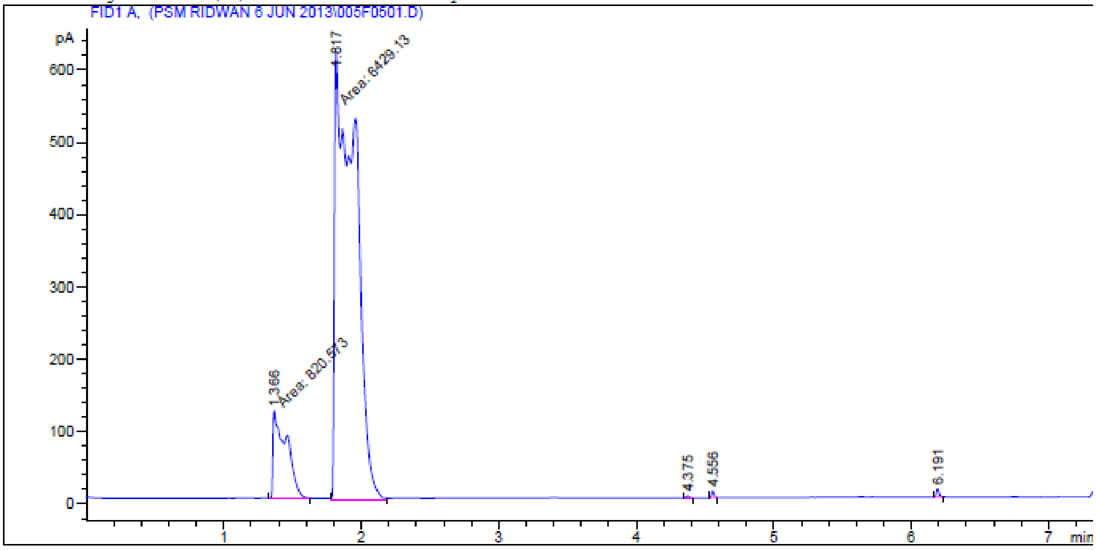
    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]     [pA]         %
    ----|-------|----|-------|----------|----------|--------|
       1   1.365 MM    0.1086  765.25470  117.45708 10.71943
       2   1.815 MM    0.1649 6319.89990  638.57581 88.52702
       3   4.366 BB    0.0233   10.50564    7.08204  0.14716
       4   4.547 BB    0.0173   18.01483   15.82313  0.25235
       5   6.188 BB    0.0212   17.07539   12.26932  0.23919
       6   7.319 BBA   0.0249    8.19974    5.49863  0.11486

    Totals :                    7138.95020  796.70601

    =====================================================================
                              *** End of Report ***
```

Data File C:\CHEM32\1\DATA\PSM RIDWAN 6 JUN 2013\005F0501.D
Sample Name: 210rpm (2) t4

===
Acq. Operator : hazimah Seq. Line : 5
Acq. Instrument : Instrument 1 Location : Vial 5
Injection Date : 6/6/2013 6:35:03 PM Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed : 6/6/2013 5:43:30 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed : 6/5/2013 11:37:42 AM by hazimah



===
Area Percent Report
===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.366	MM	0.1133	820.57257	120.68597	11.27182
2	1.817	MM	0.1730	6429.12793	619.41388	88.31391
3	4.375	BB	0.0219	3.97624	2.73948	0.05462
4	4.556	BB	0.0172	10.20802	9.04631	0.14022
5	6.191	BB	0.0209	15.97426	11.72235	0.21943

Totals : 7279.85902 763.60798

===
*** End of Report ***

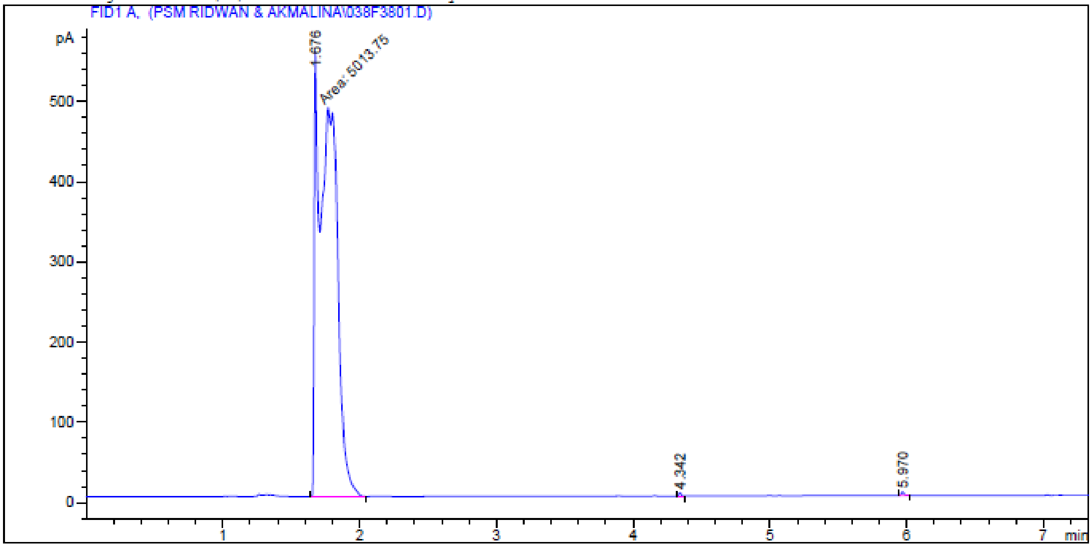
Instrument 1 6/10/2013 5:18:24 PM hazimah Page 1 of 1

OPF Juice Concentration

25% v/v

```
Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\038F3801.D
Sample Name: opf25% t0

=====================================================================
Acq. Operator   : hazimah                         Seq. Line :  38
Acq. Instrument : Instrument 1                     Location : Vial 38
Injection Date  : 6/6/2013 5:22:01 AM                   Inj :   1
                                                 Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,
```

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.676	MM	0.1504	5013.75293	555.59229	99.76196
2	4.342	BB	0.0203	5.06152	3.87067	0.10071
3	5.970	BB	0.0254	6.90176	4.14215	0.13733

Totals : 5025.71621 563.60511

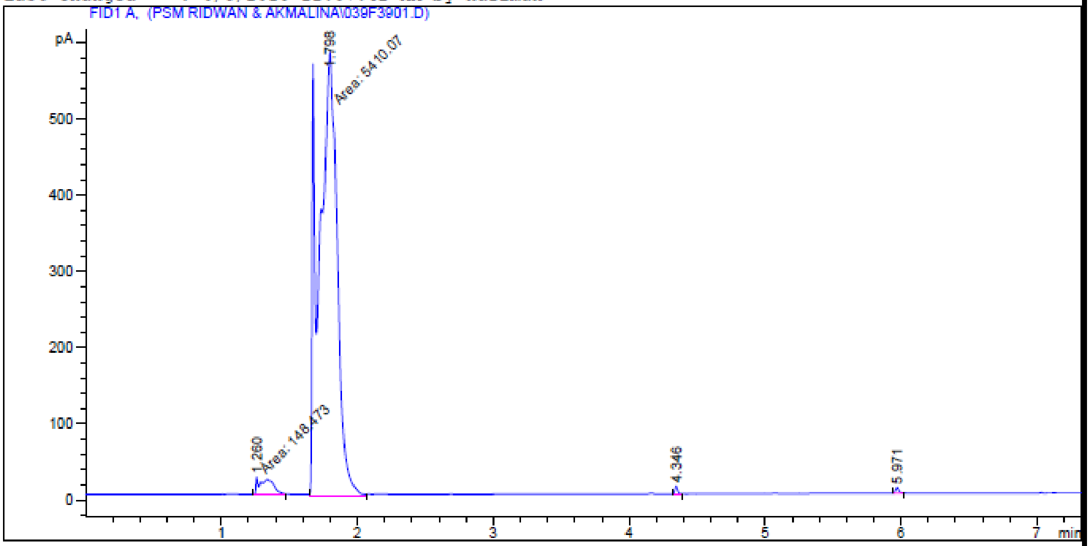
```
=====================================================================
                          *** End of Report ***
```

Instrument 1 6/10/2013 4:51:33 PM hazimah   Page   1 of 1

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\039F3901.D
Sample Name: opf25% t1

===

Acq. Operator : hazimah   Seq. Line : 39
Acq. Instrument : Instrument 1   Location : Vial 39
Injection Date : 6/6/2013 5:34:30 AM   Inj : 1
                                                              Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed : 6/5/2013 11:37:42 AM by hazimah



===

Area Percent Report

===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.260	MM	0.1094	148.47302	22.62908	2.65846
2	1.798	MM	0.1550	5410.06885	581.63989	96.86927
3	4.346	BB	0.0225	14.42088	10.16732	0.25821
4	5.971	BB	0.0246	11.95490	7.48335	0.21406

Totals : 5584.91765 621.91964

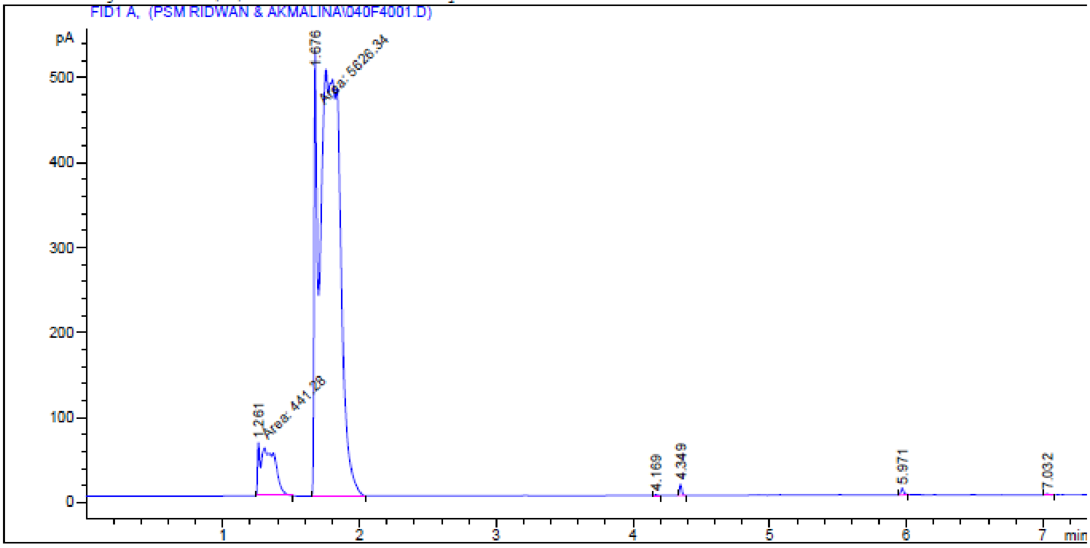
===

*** End of Report ***

Instrument 1 6/10/2013 4:54:37 PM hazimah   Page 1 of 1

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\040F4001.D
Sample Name: opf25% t2

===
Acq. Operator : hazimah Seq. Line : 40
Acq. Instrument : Instrument 1 Location : Vial 40
Injection Date : 6/6/2013 5:47:08 AM Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed : 6/5/2013 11:37:42 AM by hazimah



===
Area Percent Report
===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.261	MM	0.1195	441.28000	61.53125	7.23497
2	1.676	MM	0.1789	5626.34375	524.11176	92.24631
3	4.169	BB	0.0206	1.52637	1.14419	0.02503
4	4.349	BB	0.0191	16.07723	13.34215	0.26359
5	5.971	BB	0.0237	11.90970	7.86828	0.19526
6	7.032	BB	0.0268	2.12448	1.25078	0.03483

Totals : 6099.26153 609.24841

===
*** End of Report ***

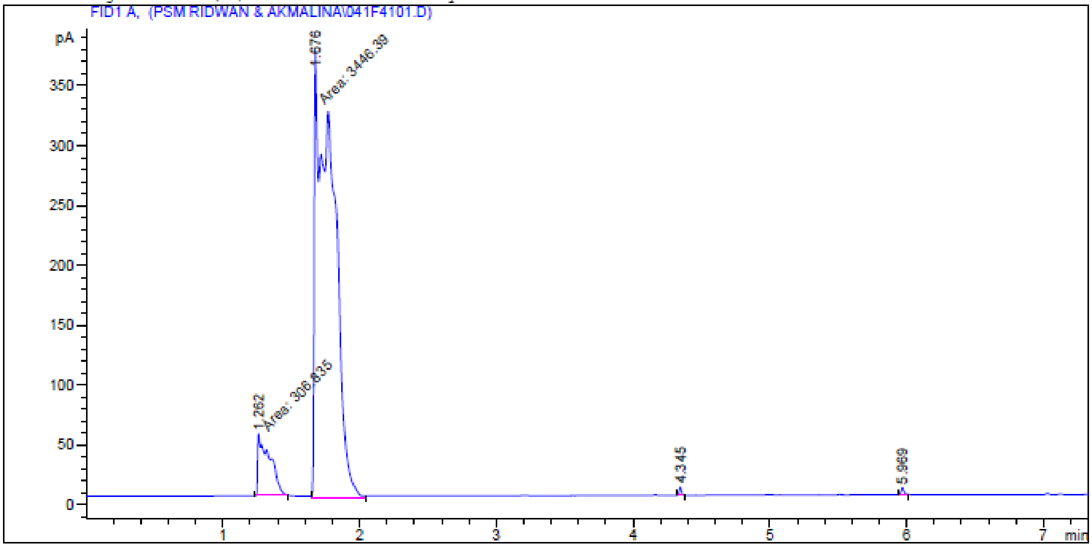
Instrument 1 6/10/2013 4:55:35 PM hazimah Page 1 of 1

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\041F4101.D
Sample Name: opf25% t3

===

Acq. Operator : hazimah Seq. Line : 41
Acq. Instrument : Instrument 1 Location : Vial 41
Injection Date : 6/6/2013 5:59:42 AM Inj : 1
Inj Volume : 1 µl

Acq. Method : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed : 6/5/2013 11:37:42 AM by hazimah



===
Area Percent Report
===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

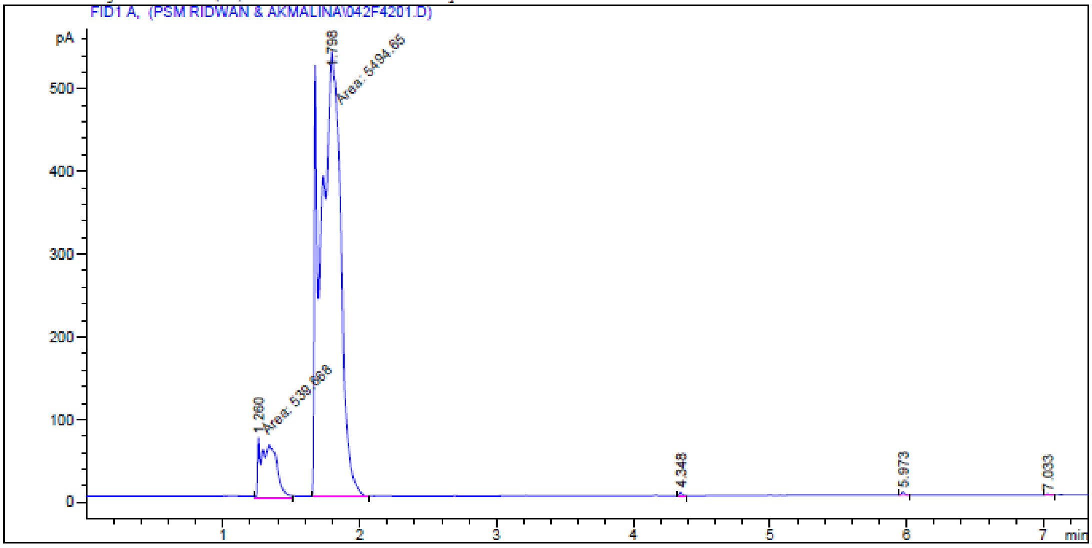
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.262	MM	0.1000	306.83511	51.13877	8.13872
2	1.676	MM	0.1542	3446.38940	372.49094	91.41457
3	4.345	BB	0.0190	8.08702	6.74937	0.21451
4	5.969	BB	0.0224	8.75439	5.87282	0.23221

Totals : 3770.06593 436.25190

===
*** End of Report ***

```
Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\042F4201.D
 Sample Name: opf25% t4

    =====================================================================
    Acq. Operator   : hazimah                        Seq. Line :  42
    Acq. Instrument : Instrument 1                    Location : Vial 42
    Injection Date  : 6/6/2013 6:12:15 AM                  Inj :   1
                                                    Inj Volume : 1 µl
    Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
    Last changed    : 6/5/2013 1:01:42 PM by hazimah
    Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
    Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A,

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]         %
    ----|-------|----|-------|----------|----------|--------|
       1   1.260 MM    0.1251  539.66785   71.92121  8.92269
       2   1.798 MM    0.1704 5494.64502  537.40332 90.84669
       3   4.348 BB    0.0196    5.04740    4.02760  0.08345
       4   5.973 BB    0.0245    6.57838    4.15570  0.10876
       5   7.033 BB    0.0258    2.32243    1.36709  0.03840

    Totals :                  6048.26107  618.87492


    =====================================================================
                             *** End of Report ***

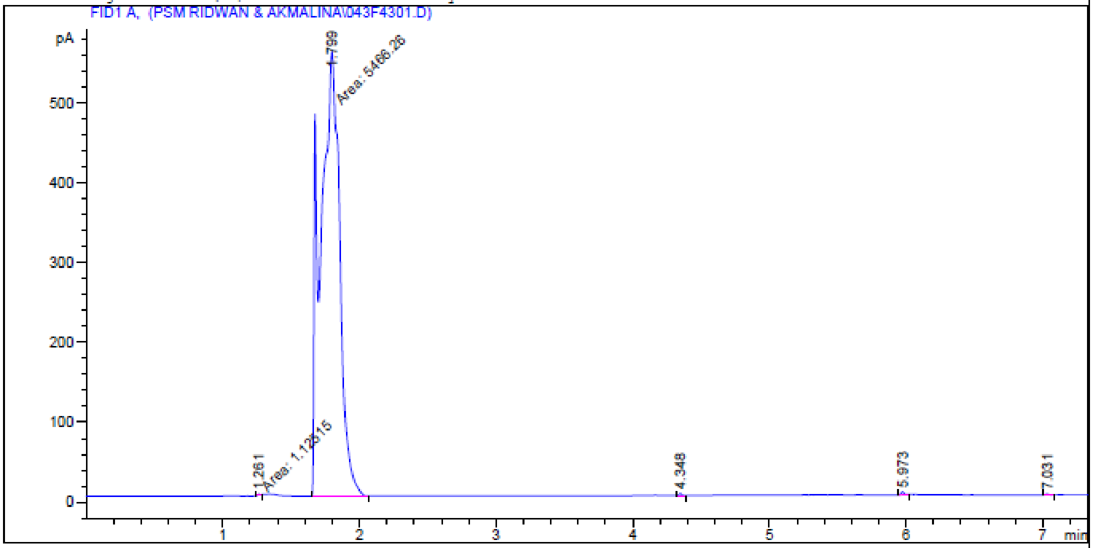

Instrument 1 6/10/2013 4:57:08 PM hazimah                           Page    1 of 1
```

50% v/v

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\043F4301.D
Sample Name: opf50% t0

===

Acq. Operator : hazimah Seq. Line : 43
Acq. Instrument : Instrument 1 Location : Vial 43
Injection Date : 6/6/2013 6:24:48 AM Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed : 6/5/2013 11:37:42 AM by hazimah



===

Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.261	MM	0.0162	1.12515	1.15748	0.02053
2	1.799	MM	0.1636	5466.25537	556.91370	99.74497
3	4.348	BB	0.0217	3.84140	2.85205	0.07010
4	5.973	BB	0.0253	6.59570	3.97772	0.12035
5	7.031	BB	0.0273	2.41419	1.38407	0.04405

Totals : 5480.23182 566.28501

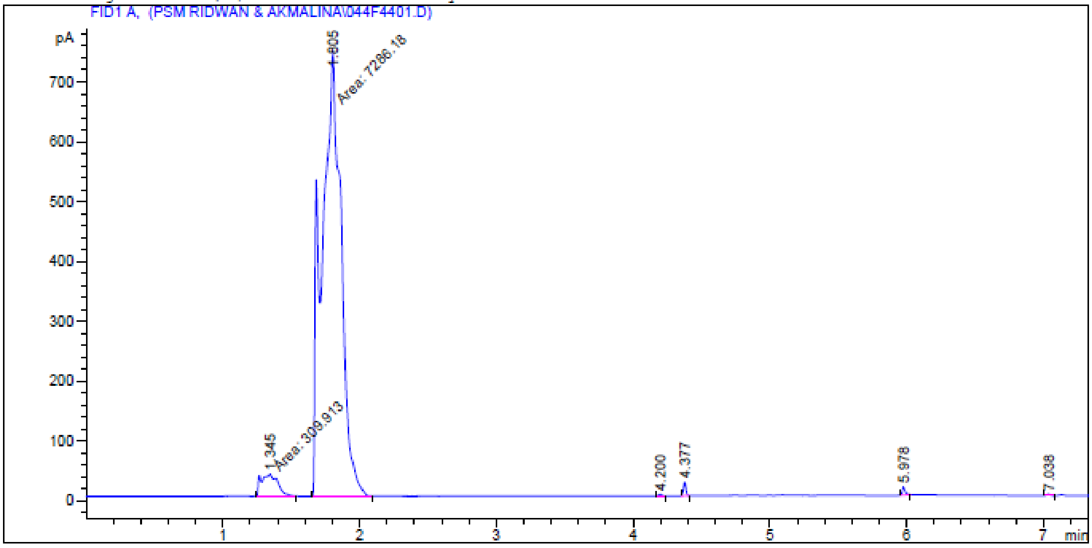
===

*** End of Report ***

Instrument 1 6/10/2013 4:58:08 PM hazimah Page 1 of 1

```
Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\044F4401.D
 Sample Name: opf50% t1

    =====================================================================
    Acq. Operator   : hazimah                        Seq. Line :  44
    Acq. Instrument : Instrument 1                    Location : Vial 44
    Injection Date  : 6/6/2013 6:37:22 AM                  Inj :   1
                                                    Inj Volume : 1 µl
    Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
    Last changed    : 6/5/2013 1:01:42 PM by hazimah
    Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
    Last changed    : 6/5/2013 11:37:42 AM by hazimah
```

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    =====================================================================
                         Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A,

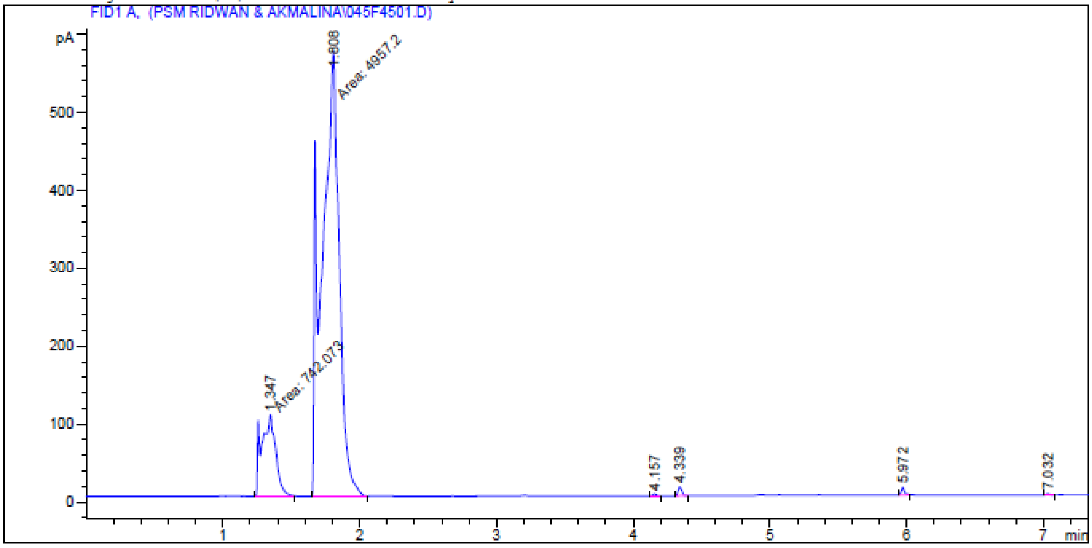
    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.345 MM    0.1392  309.91296   37.09415  4.05148
       2   1.805 MM    0.1634 7286.18066  743.27881 95.25193
       3   4.200 BB    0.0201    3.48953    2.53034  0.04562
       4   4.377 BB    0.0178   26.24658   22.24020  0.34312
       5   5.978 BB    0.0219   19.89736   13.74139  0.26012
       6   7.038 BB    0.0261    3.65114    2.22875  0.04773

    Totals :                  7649.37825  821.11364


    =====================================================================
                          *** End of Report ***
```

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\045F4501.D
Sample Name: opf50% t2

```
=====================================================================
Acq. Operator   : hazimah                        Seq. Line :  45
Acq. Instrument : Instrument 1                    Location : Vial 45
Injection Date  : 6/6/2013 6:49:54 AM                   Inj :   1
                                               Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```




```
=====================================================================
                          Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,
```

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.347	MM	0.1182	742.07294	104.60541	12.91824
2	1.808	MM	0.1449	4957.20264	570.35822	86.29652
3	4.157	BB	0.0250	3.67731	2.25337	0.06402
4	4.339	BB	0.0310	21.16263	11.20492	0.36841
5	5.972	BB	0.0274	16.85070	9.64543	0.29334
6	7.032	BB	0.0274	3.41678	1.95365	0.05948
Totals :				5744.38299	700.02099	

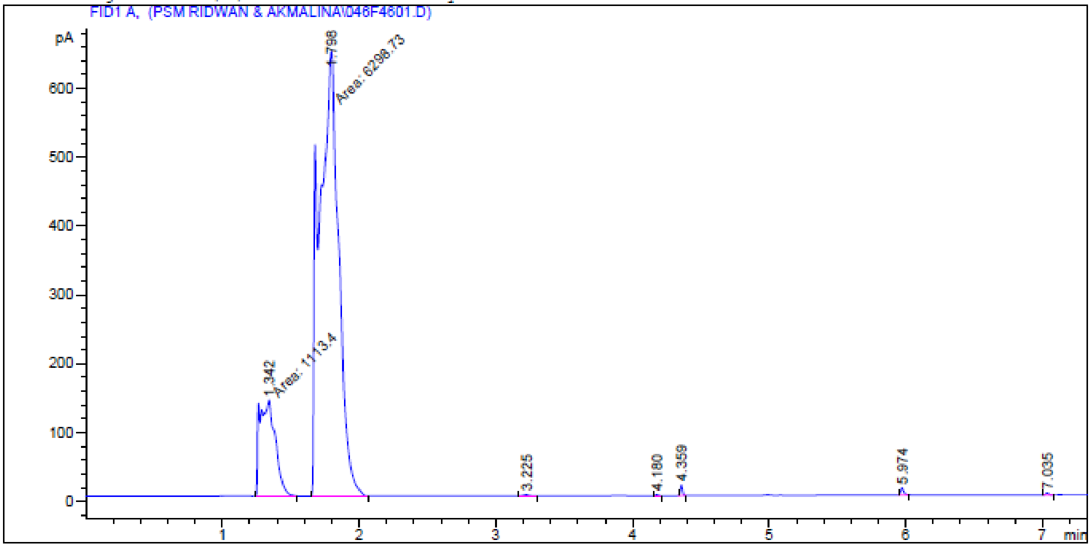
```
=====================================================================
                          *** End of Report ***
```

Instrument 1 6/10/2013 5:00:32 PM hazimah

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\046F4601.D
Sample Name: opf50% t3

===

Acq. Operator : hazimah   Seq. Line : 46
Acq. Instrument : Instrument 1   Location : Vial 46
Injection Date : 6/6/2013 7:02:29 AM   Inj : 1
                                         Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed : 6/5/2013 11:37:42 AM by hazimah



===

Area Percent Report

===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.342	MM	0.1329	1113.40125	139.63094	14.92744
2	1.798	MM	0.1625	6298.73486	645.99609	84.44752
3	3.225	BB	0.0462	5.04350	1.67240	0.06762
4	4.180	BB	0.0203	2.30393	1.75967	0.03089
5	4.359	BB	0.0173	17.46001	15.35751	0.23409
6	5.974	BB	0.0240	16.35303	10.60637	0.21925
7	7.035	BB	0.0269	5.45987	3.20158	0.07320

Totals : 7458.75645 818.22457

===

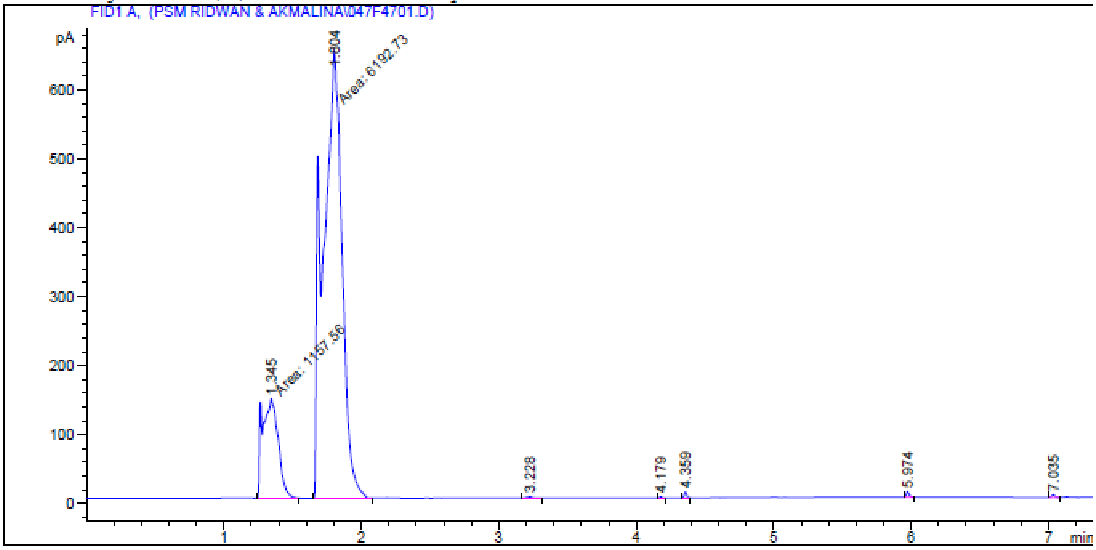
*** End of Report ***

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\047F4701.D
Sample Name: opf50% t4

===

Acq. Operator   : hazimah   Seq. Line : 47
Acq. Instrument : Instrument 1   Location : Vial 47
Injection Date : 6/6/2013 7:15:01 AM   Inj :   1
                                                  Inj Volume : 1 µl

Acq. Method   : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed   : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed   : 6/5/2013 11:37:42 AM by hazimah



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Area Percent Report

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Sorted By   :   Signal
Multiplier   :   1.0000
Dilution   :   1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.345	MM	0.1335	1157.55774	144.56352	15.67290
2	1.804	MM	0.1593	6192.73291	648.10645	83.84730
3	3.228	BB	0.0482	5.41230	1.79362	0.07328
4	4.179	BB	0.0208	1.79069	1.32542	0.02425
5	4.359	BB	0.0190	9.97644	8.32898	0.13508
6	5.974	BB	0.0225	12.12669	8.09431	0.16419
7	7.035	BB	0.0260	6.13022	3.77073	0.08300
Totals :				7385.72699	815.98302	

===

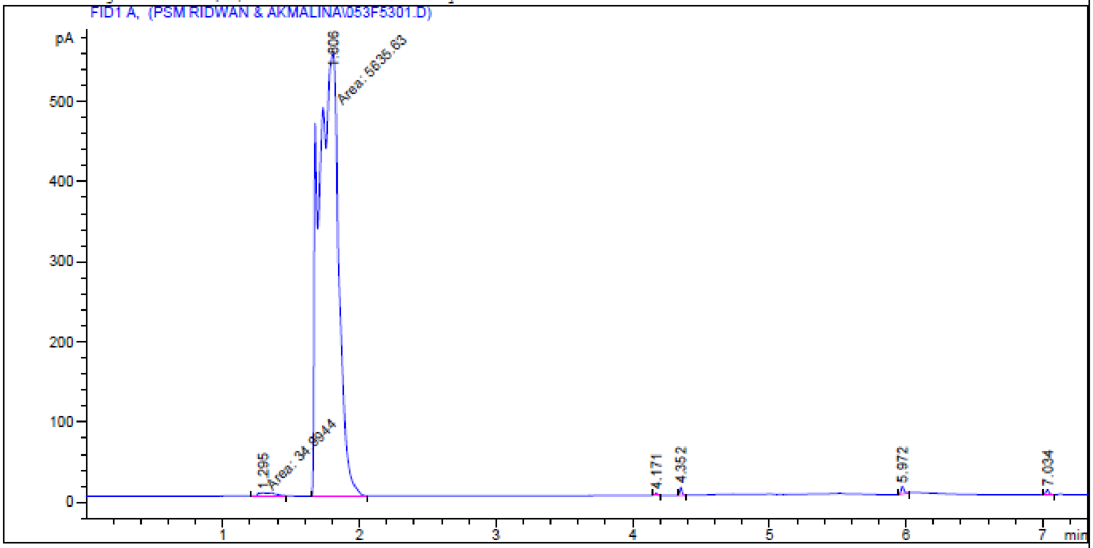
*** End of Report ***

100% v/v

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\053F5301.D
Sample Name: opf100% t0

===

Acq. Operator : hazimah   Seq. Line : 53
Acq. Instrument : Instrument 1   Location : Vial 53
Injection Date : 6/6/2013 8:30:16 AM   Inj : 1
                Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed : 6/5/2013 11:37:42 AM by hazimah



===
Area Percent Report
===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

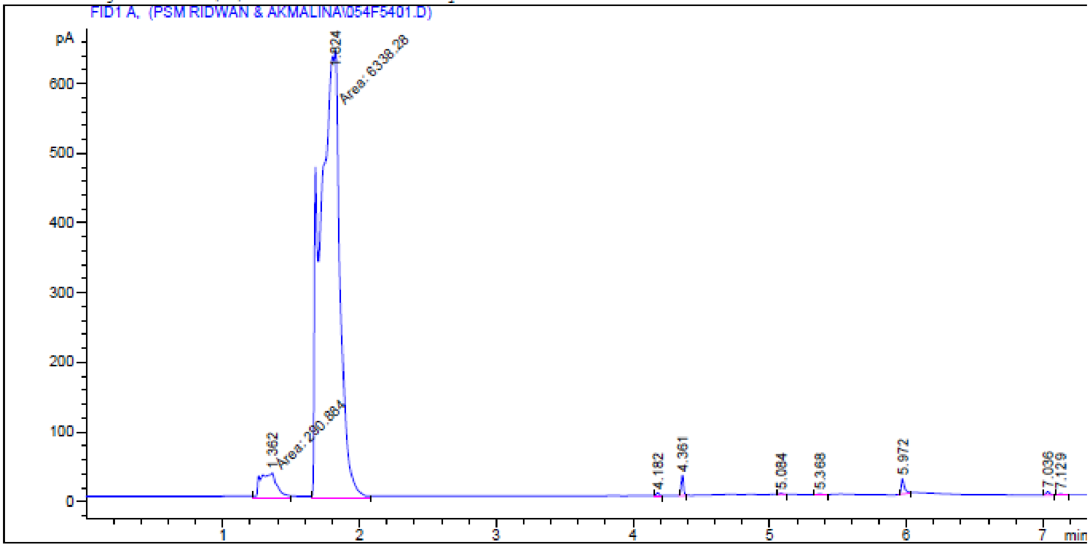
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.295	MM	0.1383	34.99438	4.21745	0.61266
2	1.806	MM	0.1696	5635.63232	553.85931	98.66497
3	4.171	BB	0.0203	4.06253	3.10550	0.07112
4	4.352	BB	0.0188	11.42020	9.67028	0.19994
5	5.972	BB	0.0251	15.31547	9.37162	0.26813
6	7.034	BB	0.0269	10.46296	6.13080	0.18318
Totals :				5711.88787	586.35495	

===
*** End of Report ***

Instrument 1 6/10/2013 5:08:07 PM hazimah   Page 1 of 1

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\054F5401.D
Sample Name: opf100% t1

===
Acq. Operator   : hazimah   Seq. Line : 54
Acq. Instrument : Instrument 1   Location : Vial 54
Injection Date : 6/6/2013 8:42:50 AM   Inj :   1
                                                Inj Volume : 1 µl
Acq. Method   : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed   : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed   : 6/5/2013 11:37:42 AM by hazimah



===
Area Percent Report
===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

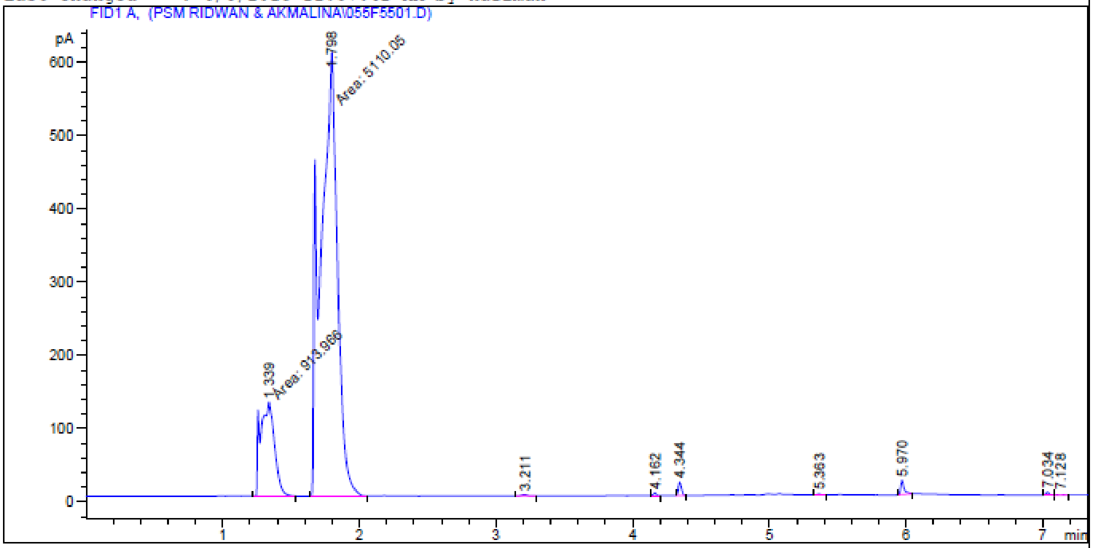
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.362	MM	0.1372	290.88416	35.34396	4.32860
2	1.824	MM	0.1651	6338.27783	639.84021	94.31890
3	4.182	BB	0.0205	5.30133	4.00166	0.07889
4	4.361	BB	0.0181	32.18876	28.72357	0.47900
5	5.084	BB	0.0232	2.66149	1.70745	0.03961
6	5.368	BB	0.0296	2.23889	1.27009	0.03332
7	5.972	BB	0.0245	36.56400	21.86549	0.54410
8	7.036	BB	0.0268	8.76662	5.16679	0.13045
9	7.129	BB	0.0294	3.16725	1.65259	0.04713
Totals :				6720.05033	739.57181	

===
*** End of Report ***

Instrument 1 6/10/2013 5:08:51 PM hazimah   Page 1 of 1

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\055F5501.D
Sample Name: opf100% t2

===
Acq. Operator   : hazimah   Seq. Line : 55
Acq. Instrument : Instrument 1   Location : Vial 55
Injection Date : 6/6/2013 8:55:23 AM   Inj :   1
                                                Inj Volume : 1 µl
Acq. Method   : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed   : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed   : 6/5/2013 11:37:42 AM by hazimah



===
Area Percent Report
===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.339	MM	0.1186	913.96643	128.40797	14.95668
2	1.798	MM	0.1405	5110.04639	606.26837	83.62377
3	3.211	BB	0.0430	5.10819	1.91946	0.08359
4	4.162	BB	0.0216	5.41976	3.81545	0.08869
5	4.344	BB	0.0239	28.55577	18.65377	0.46730
6	5.363	BB	0.0275	2.03262	1.21741	0.03326
7	5.970	BB	0.0273	35.19987	19.27579	0.57603
8	7.034	BB	0.0279	7.43303	4.15721	0.12164
9	7.128	BB	0.0310	2.99647	1.45812	0.04904
Totals :				6110.75853	785.17356	

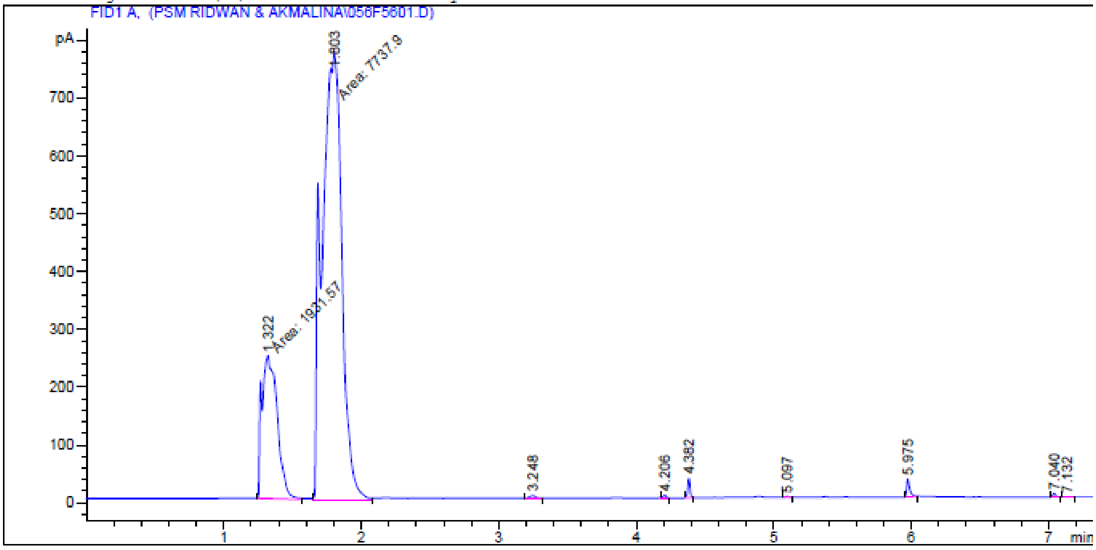
===
*** End of Report ***

Instrument 1 6/10/2013 5:09:45 PM hazimah   Page   1 of 1

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\056F5601.D
Sample Name: opf100% t3

===

Acq. Operator : hazimah Seq. Line : 56
Acq. Instrument : Instrument 1 Location : Vial 56
Injection Date : 6/6/2013 9:08:17 AM Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed : 6/5/2013 11:37:42 AM by hazimah



===

Area Percent Report

===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.322	MM	0.1303	1931.57349	247.15585	19.72859
2	1.803	MM	0.1666	7737.89990	774.17712	79.03289
3	3.248	BB	0.0415	11.77638	4.09859	0.12028
4	4.206	BB	0.0198	6.51421	4.81839	0.06653
5	4.382	BB	0.0171	37.10036	33.16684	0.37893
6	5.097	BB	0.0199	2.12236	1.66288	0.02168
7	5.975	BB	0.0230	47.53342	30.89248	0.48549
8	7.040	BB	0.0256	12.27291	7.30770	0.12535
9	7.132	BB	0.0285	3.94129	2.14016	0.04026

Totals : 9790.73432 1105.42003

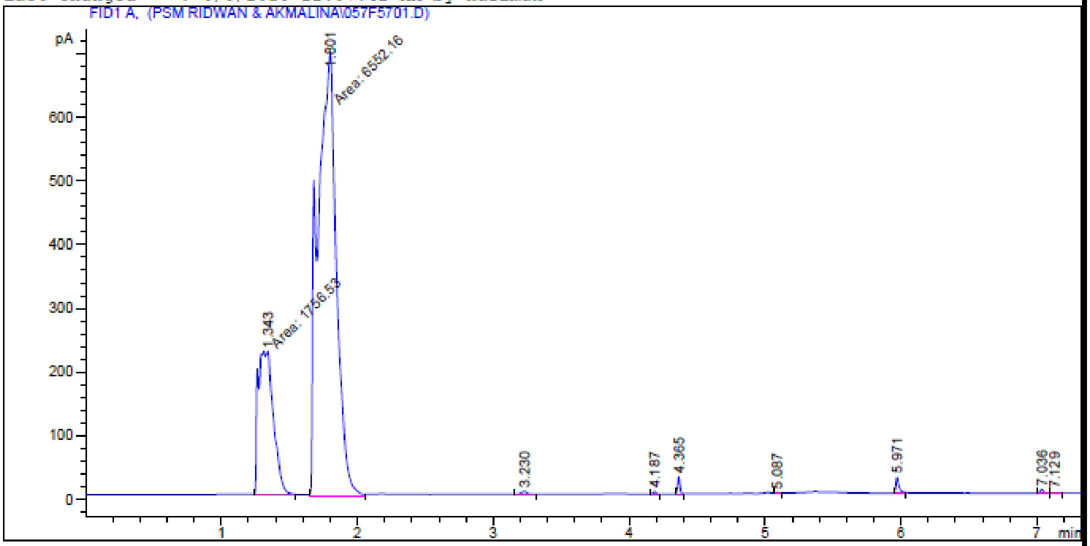
===

*** End of Report ***

Data File C:\CHEM32\1\DATA\PSM RIDWAN & AKMALINA\057F5701.D
Sample Name: opf100% t4

===

```
Acq. Operator   : hazimah                        Seq. Line :  57
Acq. Instrument : Instrument 1                    Location : Vial 57
Injection Date  : 6/6/2013 9:20:45 AM                  Inj :   1
                                               Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\STIP ANALYSIS IMPROVED EDITED.M
Last changed    : 6/5/2013 1:01:42 PM by hazimah
Analysis Method : C:\CHEM32\1\METHODS\B4 SHUTDOWN.M
Last changed    : 6/5/2013 11:37:42 AM by hazimah
```



===
Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	1.343	MM	0.1302	1756.53137	224.76987	20.89177
2	1.801	MM	0.1568	6552.15674	696.51495	77.92982
3	3.230	BB	0.0456	11.12538	3.97897	0.13232
4	4.187	BB	0.0203	4.25942	3.24963	0.05066
5	4.365	BB	0.0180	29.84866	26.72527	0.35501
6	5.087	BB	0.0166	1.23013	1.14241	0.01463
7	5.971	BB	0.0232	38.07273	24.46796	0.45283
8	7.036	BB	0.0281	11.13855	6.47643	0.13248
9	7.129	BB	0.0281	3.40296	1.87898	0.04047
Totals :				8407.76595	989.20447	

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*** End of Report ***

Instrument 1 6/10/2013 5:12:15 PM hazimah   Page   1 of 1

TECHNICAL REPORT

Potential Evaluation Oil Palm Frond Juice for Bioethanol Fermentation

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ABSTRACT: The fossil fuels degrade abundantly since 21st century. The usages of fossil fuels tend to emit the greenhouse gas that effect to global warming as well human health. To overcome these problems, the bioethanol introduced by the researches to substitute the fossil fuels. Bioethanol is a bioenergy produced by yeast fermentation at several conditions compared to conventional fossil fuels. Bioethanol is being studied because the unique properties and renewable fuels. The production of bioethanol in industrial scale involved high cost due to the usage of foods as sugar raw materials. Thus, this research is to evaluate the oil palm frond (OPF) juice as raw material for bioethanol fermentation. In this study, the bioethanol produces in shake flask by using *Saccharomyces cerevisiae* and OPF juice as medium component. Three parameters study are pH, agitation speed, and OPF juice concentration. The best condition of parameters determine by the highest ethanol concentration produce. Inoculum prepare in shake flask to cultivate the yeast in medium growth and ferment in 500 ml shake flasks containing medium growth and beef extract. The flasks incubate in 48 hours, 12 hours interval for sample withdraw. The products analyse for sugar concentration and bioethanol concentration. The results for the best condition in bioethanol fermentation are in pH 7, rpm 150 and 100% volume percentage of OPF juice concentration

Key word: oil palm, frond, ethanol production, GC, *S. cerevisiae*

1. INTRODUCTION

The fossil fuels are non-renewable. They are limited in supply and will one day be depleted. There is no escaping this conclusion. Fossil fuels formed from plants and animals that lived hundreds of millions of years ago and became buried way underneath the Earth's surface where their remains collectively transformed into the combustible materials we use for fuel. For example, petroleum is the source of about 170 quadtrillion BTUs or quad of energy of more than 460 quads the world uses, far more than derived from other sources [26].

The negative global warming impact of fossil fuels, volatile oil price and political unstable in oil exporting countries resulted in a significant increase in international interest in alternative fuels and led policy makers in the world to issue ambitious goals for substitution of alternative for convention fuels [26]. Besides that, the great dangers posed to natural ecosystems that result from collecting fossil fuels, particularly coal and oil. Oil spills have devastated ecosystems and coal mining has stripped lands of their vitality. This is the primary reason to discontinue the pursuit to tap the vast oil reserves in the Arctic National Wildlife Refuge (ANWR).

In other to reduce the side effects of fossil fuels, the alternative energy is the best choice. One of the alternative energy is bioenergy, produced from the biological process. Examples of sources of bioenergy include wood and sawmill waste, charcoal, biogas resulting from the anaerobic decomposition of waste, as well as liquid biofuels, such as bioethanol and biodiesel. Bioethanol made biologically by fermentation from a variety of biomass sources is widely recognized as a unique transportation fuel and original material of various chemical with powerful economic, environmental and strategic attributes [26]. Bioethanol (ethyl alcohol, grain alcohol, CH_3-CH_2-OH or EtOH) is a liquid biofuel which can be produced from several different biomass feedstocks and conversion technologies [1]. Bioethanol is an attractive alternative because it is a renewable biobased resource and it is oxygenated thereby provides the potential to reduce particulate emissions in comparison ignition engines. The properties of ethanol are shown in and has a higher octane number, broader flammability limits, higher flame speeds and higher heats of vaporisation than gasoline.

Bioethanol is the most widely used liquid biofuel. The largest producers in the world are United States, Brazil, and China. Production of bioethanol from sugar cane in Brazil accounted nearly 18% of the country's automotive fuel needs in 2004 [15]. In 2006, global production of bioethanol reached 13.5 billion gallons up to from 12.1 billion gallons in 2005 [1]. World ethanol production (all grades) has been reached a record 62×10^9 litre in 2007, with the United States and Brazil as dominant producers (approximately 70%) [licht]. In 2009, the US produced 39.5×10^9 litres of ethanol using corn as a feedstock while the Brazil created about 30×10^9 litres of ethanol using sugar cane [15]. [1] About 90% of bioethanol is derived from corn in United States and all the Brazil's bioethanol is produced from sugar cane and cited that any country with a significant agronomic based economy can used current technology for bioethanol fermentation due to technology for bioethanol production from non-food plant sources has been developed to the point at which large scale production will be reality in the next few years [8].

The studies of bioethanol is become interest in researchers due to the high demand in worldwide. The production of fermentation by using sugar from sugar canes, grains, sweet potatoes and corn-starch are been commercialised. Since the sugar source is come from the food starch, the cost of the fermentation is high. The use of renewable resource such as agricultural and agro based-industry wastes as raw material for the fermentable sugars can help to reduce the production cost and the dependence on food crops [24]. Thus, the raw material used in this study is wastes based from oil palm industry which is oil palm frond.

Oil palm contains abundant of sugar composition in all of the parts. [20] The oil palm trunk (OPT) is contains higher sugar composition rather other part of oil palm. Thus, the OPT is the main raw material for ethanol fermentation. The OPT sap been hydrolysed to obtain sugar i.e. glucose and xylose [24] but recently the sap is simply pressed [6]. This step is simple compared to OPT sap hydrolyse.

In addition, the composition of sugar in oil palm frond (OPF) is similar as the sugar composition in OPT. The production of sugar from dried OPF fibre involving the conversion of cellulose and hemicellulose into glucose and xylose through hydrothermal treatment followed by enzymatic hydrolysis, use of high temperature and pressure, and also cellulose enzymes. However, the renewable sugars can be obtained from OPF simply by pressing the fresh OPF to obtain the juice [24].

The comparison of sugar concentration between OPF juice [24] and oil palm trunk (OPT) sap [6]. The amount of sugars in OPF juice is 76.09±2.85 g/l. Glucose was the major sugar component in OPF juice at 53.59±2.86 g/l, sucrose at 20.46±1.56 g/l and fructose at 1.68±0.75 g/l. Meanwhile, the sugar composition in OPT sap is 59.5±5.6 g/l. Similar to OPF juice, glucose was the major component in OPT sap at 50.2±2.8 g/l, followed by sucrose at 3.8±0.5 g/l and fructose at 3.1±1.3 g/l and the other sugar composition shown in Table 6. The presence of sugar in OPF and OPT was expected due to the photosynthesis process.

Therefore, the evaluation of ethanol potential from fermentation of OPF juice is sufficient. The objectives of this research are to study the effect of process parameters (pH, agitation speed and OPF juice concentration) for the production of bioethanol from fermentation using OPF juice and to evaluate the best condition for the production of bioethanol in term of yield and productivity from fermentation using OPF juice as renewable and sustainable substrate.

In order to achieve the stated objectives, scopes have been identified are the evaluation of the feasibility of OPF juice as a direct substrate for bioethanol production by using yeast *Saccharomyces cerevisiae*, three main factor affecting bioethanol productions from literature studies are using lab scale experiment, which are initial pH, agitation rate, and percentage of OPF juice, and the identification of the ethanol yield and productivity of each parameter given.

2. MATERIALS AND METHODS

2.1 Bacterial strain

The strain employed throughout these studies is an industrial sake brewing strain, *Saccharomyces cerevisiae* Kyokai no. 7 (ATCC 26422) obtained from the American Type Culture Collection. The strain is maintained by regular sub culturing on YPD agar medium (in g/l: agar 20, yeast extracts 1.5, peptone 1.5 and dextrose 20) every five weeks. The strain incubated in 250 ml of 500 ml flask contain of medium (enriched with NaCl 5 g/l, peptone 5 g/l, yeast extracts 1.5 g/l) and vented with cotton wool to allow aeration for 12-18 hours at 30 ^{O}C and 150 rpm. This inoculums preparation applied in all parts of the experiment.

2.2 Fermentation of Ethanol

Ethanol is carried out through one-stage cultivation fermentation in shake flasks. OPF juice in this study obtained by pressing the fresh OPF following by the method described earlier. For the study of initial pH effect, the initial pH of the medium (sodium chloride 5 g/l, peptone 5 g/l, beef extracts 1.5 g/l and yeast extracts 1.5 g/l). and the OPF juice adjust at pH value of 4, 6, 7, and 8 using 1M of hydroxide acid and 1M of sodium hydroxide. Another set of experiment is to adjust the OPF juice concentration via volume percentage of 25%, 50% and 100%. Meanwhile, the effect of agitation speed is adjust at 150 rpm, 180 rpm, and 210 rpm. The culture incubated in 30ºC under anaerobic condition. The initial pH effect and agitation speed conducted in duplicated. The sample collected in 12 hours interval for 36 hours.

2.3 Instrumentation Setup

Gas chromatography Agilent Technologies (6890 Series) equipped with a flame ionization detector (FID) was set up to determine ethanol concentrations. The column used was HP-INNOWax Polyethylene Glycol (30 m x 250 µm x 0.25 µm nominal). The oven temperature and injector and detector temperature are 70 °C and 250 ºC respectively. Initial temperature 70 °C held for 4 min, rate of increase 20 °C/min until 120 °C min held for 2 min). The carrier gas was helium at a flow rate of 45 ml/min.

2.3 Analytical Procedure

2.3.1 Ethanol Determination. *n*-propanol (1 %v/v) has been used as solvent or internal standard in all samples. Internal standard is being used as a reference standard for normalization of ethanol peak and also to mark the peak of ethanol in the chromatogram by relative retention time. It is previously diluted from 99.8 %v/v of HPLC grade *n*-propanol. For external standard, ethanol prepares in 2-10 %v/v of concentration using diluted 99.98 %v/v of HPLC grade ethanol. For GC-FID sample, Samples are prepared in the vial by filtering with 0.20 µm nylon filter. Peak of ethanol observed at R_t =2.985 min convert to the concentration term (g/l) using previous obtained standard curve.

3. RESULT AND DISCUSSION

3.1 Effect of Initial pH

The effect of different pH on ethanol production using OPF juice by *S. cereviasie* shown in Figure 1. The highest ethanol production is in initial pH of 7, up to 29.665 g/L.

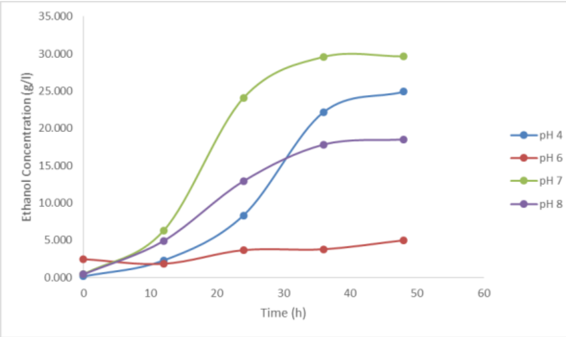

Figure 5: Ethanol profile from OPF juice fermentation based on initial pH.

Table 5: Effect of pH on ethanol production from OPF juice.

Time	Initial pH			
(h)	4	6	7	8
0	0.147	2.473	0.427	0.425
12	2.290	1.843	6.267	4.901
24	8.339	3.680	24.097	12.932
36	22.185	3.799	29.596	17.832
48	24.922	4.996	29.665	18.530

This followed by pH 4, pH 8 and pH 6 which is 24.992 g/L, 18.350 g/L, and 4.996 g/L respectively. Table 1 shows the other result in varies pH values according to 12 hours interval in 48 hours incubated. This current study agreed with other studies reported that yeast grows and fermentation perform the best in natural or slightly acidic environment [13]; [9].

The most suitable initial pH for bioethanol production is in pH 4 [5]. This statement supported by other studies that mentioned the highest bioethanol production is in pH 4 for initial pH of medium [14]; [18]; [21]. The optimum pH value for *S. cerevisiae* is in range of 4.2 to 4.8 [2]. However, this statement is contradict with the result shown due to indifference between the fermentation process conducted at pH 5.4 and pH 4.7 [16} supported by the insignificant difference between fermentation yields conducted in pH range between 2.4 to 8.6 [22].

The bioethanol production high when the pH value is lower. The lower bioethanol productivity may be due to the lower metabolic rate of cell yeast used [10]. The increasing of pH value in the molasses hydrolysate will also reduce alcohol hydrogenase activity in *S. crevisiae* cell and lead to the formation of acidic acid instead of bioethanol in the process [11]. The different strain also contribute the optimum pH for bioethanol production.

3.2 OPF Juice Concentration

OPF juice is the sugar raw of the fermentation. In other words, this factor also means the effect sugar concentration in the ethanol production. [3] The higher glucose concentration leads the higher concentration of bioethanol produced. Thus, the highest ethanol production is obtain in 100% of OPF juice concentration which is 45.365 g/L t interval of 36 hour. This followed by 50%, and 25% of OPF juice concentration which are 26.319 g/L and 12.017 g/L respectively. Table 1 shown the ethanol production for OPF juice concentration values based on 12 hours interval for 48 hours incubated.

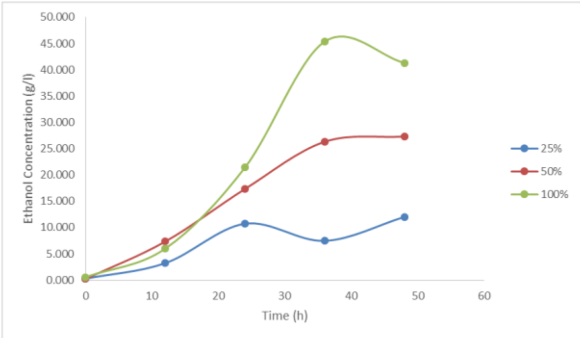

Figure 6: Ethanol profile for OPF juice fermentation based on OPF juice fermentation.

Table 6: Effect of OPF juice concentration on ethanol production from OPF juice.

Time	OPF juice concentration (%)		
(h)	25	50	100
0	0.275	0.215	0.569
12	3.221	7.329	5.982
24	10.722	17.333	21.420
36	7.470	26.319	45.363
48	12.017	27.312	41.307

From the ethanol profile in Figure 2, the ethanol production increased due to the increasing of OPF juice concentration since the presence of the sugar composition in OPF juice [24]. This supported by the increasing of fermentable sugars content from 5 to 13% (w/w), bioethanol yields increase for both applied substrates (thin juice and molasses) [17]. The maximum bioethanol production (% v/v) was 8.5 for V-1 and 9.5 for V-2 due to the different composition of carbon source for both V-1 and V-2 [4].

3.3 Agitation Speed

The increasing of agitation speed also affected the production of ethanol. The highest ethanol produced is 150 rpm of agitation speed, with a maximum ethanol concentration of 27.792 g/L at interval of 36 hour. This followed by 180 rpm and 210 rpm with ethanol concentration of 19.735 g/L and 19.212 g/L at interval of 24 hour respectively.

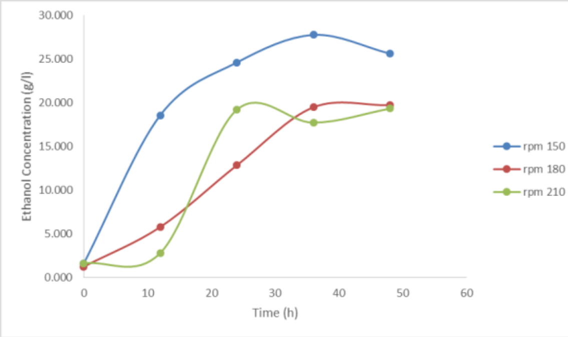

Figure 7: Ethanol profile of OPF juice fermentation according to agitation speed.

Table 7: Effect of agitation speed on ethanol production from OPF juice.

Time (h)	Agitation speed (rpm) 150	180	210
0	1.584	1.228	1.603
12	18.587	5.771	2.791
24	24.632	12.842	19.212
36	27.792	19.495	17.737
48	25.654	19.735	19.348

The effect of the agitation speed from 50 rpm to 150 rpm [5]. Thus, this statement is in agreement with the bioethanol production yield using mechanical agitation was higher than without or lower agitation speed [12]. However, this study consist the agitation speed above 150 rpm. Since the OPF juice concentration is constant, the viscosity of medium is constant and unaffected to bioethanol production. So, the increasing of agitation speed above to 150 rpm reduced the bioethanol production due to the turbulence of medium will create the shear force that cause the cells break down. The batch fermentation of wood hydrolysate by *Pichia stipitis* indicated the highest bioethanol production was obtain in 150 rpm with 0.37 g/g glucose after 72 hours fermentation [19].

4. CONCLUSION

The OPF contain the composition of sugar or carbon source similar as OPT sap. So, the OPF also be able to become raw material of the bioethanol fermentation. The OPF juice obtained from the pressing the OPF and proceed to the further research.

In this research, the parameters used are pH, agitation speed and OPF juice concentration. The bioethanol production is highest in the yeast natural or slightly acidic and decrease when more acidic of more alkali, the suitable agitation speed and the highest OPF juice concentration. Thus, the best condition of the bioethanol fermentation is in pH 7 of initial pH value, agitation speed of 150 rpm, and 100% v/v OPF juice concentration.

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